PROTOZOA AND DISEASE

J. JACKSON CLARKE

PART IV. RHIZOPOD PROTOZOA

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THE CAUSE OF CANCER
AND OTHER DISEASES

BEING

PART IV. OF

"PROTOZOA AND DISEASE"


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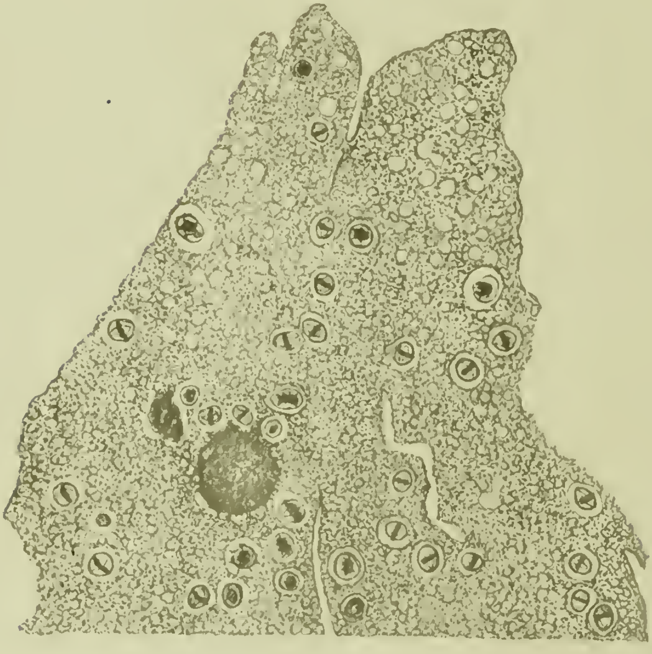
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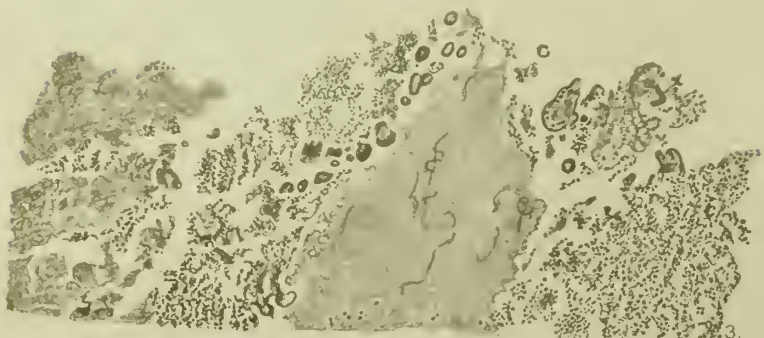
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FRONTISPIECE

STAGES OF DIDYMIUM IN STAINED SECTIONS

Fig. 1.—A part of a plasmodium passing into the sporangium stage. The protoplasmic mass is divided by numerous folds and clefts, and it contains numerous nuclei and larger chromidial pools. The supporting framework consists of a central reticulated part from which typical capillitium fibres pass towards the protoplasmic mass and a thinner marginal part resembling a basement membrane. In this section the so-called vessels are seen not to be special structures, but merely clefts or spaces between the folds of the plasmodium. Drawing eye-piece. × 100 diams.

Fig. 2.—Part of the same plasmodium (Fig. 2) shown under a high power: numerous small nuclei are in the equatorial stage of division. Drawing eye-piece. × 800 diams.

Fig. 3.—Part of a yellow nodule. Hyaline material is breaking up into nucleated amœbulæ, as shown under a higher power in Fig. 28. The structure has a momentous bearing on the interpretation to be placed on certain pathological formations, such as protozoan cystic ureteritis, etc. Drawing eye-piece. × 100 diams.

RHIZOPOD PROTOZOA

THE CAUSE OF CANCER AND OTHER DISEASES

BEING

PART IV. OF "PROTOZOA AND DISEASE"

BY

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TO

L. J. C.

PREFACE

Until the present year I have been unable to point to any particular subdivision of the protozoa and say, to this or that class belong the protozoa of cystic ureteritis, cancer, molluscum, etc. Observations recorded in the first five chapters of this book have enabled me to state definitely that they belong to the same group of organisms as the mycetozoa.

With regard to molluscum I give a detailed account of vital processes, so that any one, who will take the trouble to obtain the material, and who is accustomed to observe living protozoa, can know that the bodies characteristic of that disease are protozoa akin to mycetozoa.

In all the original representations of stationary objects here reproduced I have ensured absolute objectivity by drawing the main features of every structure referred to by means of a mechanical drawing apparatus.

J. JACKSON CLARKE.

London,
September, 1915.

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PROTOZOA AND DISEASE

CHAPTER I

INTRODUCTION

A TIME of national peril and widespread personal loss may not appear to all to be appropriate for the publication of a book dealing with the causes of disease. But when we consider the irreparable loss among our best and bravest, it is plain that our profession must be more than ever careful of the physical welfare of those who remain, and in order to be efficient it must watch closely all that throws light on the causes of disease.

The war against disease is one in which all nations are allied. In civilised countries the medical profession is the standing army for this war.

Although no peace is sought, no truce with disease, compromise such as we have in vaccination is sometimes the best we can do for humanity until wider knowledge may make it unnecessary to stoop to compromise. Our country is now on its trial; its biological merits are being tested by the crudest operations of the instinct of self-preservation exerted against it. If the Empire is not able to overcome its foes none of its true children will wish to survive. The survival of an empire depends on the biological efficiency of the average individual. *Homo sapiens* is the name given by biology to man, and it seems probable that no one who has reached middle life can avoid some feeling of being flattered when he reads his biological name. This sense of flattery fades when we realise that all varieties of man constitute but one species, and that the term applies to the Australian black equally with the European white. What, we may ask, is the fundamental element in biological efficiency? It consists in the capacity to adjust ourselves to our environment. To do this in our professional life we must know all we can of the causes of disease, and in national life we must know what is going on in neighbouring states. Have we as a nation been kept informed of the doings and designs of our neighbours? As a nation we were not awake to the designs against us. For this what may

be termed the committee of management of the Empire was not free from blame. And nine months after the storm broke it was found that some official minds concerned with munitions had lost elasticity, as official minds after long years of routine work are prone to do. Are we in our narrower professional body free from the failings of the commonwealth at large? Of this we can judge only by the results of our campaigns against disease. One of these was an imperial expedition against cancer. Our campaign began some twelve years ago. What has the issue been? There is but one word for it—failure. Must the greater imperial war end like that? *Absit omen!* But there is hope. In the committee of management of the Empire there have been changes: eliminations and assimilations. In that of Imperial Cancer Research there were no such changes. In the Empire public opinion has some force. In our profession public opinion consists of the beliefs of the average medical man. The foundation of current opinion in the profession consists chiefly in instruction imbibed by medical students from clinical teachers, and to understand what this is like in an average case I take a relevant passage from the first clinical lecture that comes to hand: it is by a man I know and respect highly as a citizen and as a clinician:—

"Its causation is very obscure. I myself

have not seen a case of molluscum contagiosum for a year, and now I get these two cases brought before me on the same day. The man, you see, has three umbilicated lesions on the forehead, one of which I have treated in order to show the molluscum bodies. The diagnosis is easy, especially by the microscope. All you have to do is to incise one of the lumps, and express the matter, and you get a substance like candle wax. This appears lobulated to the naked eye, but when teased out with caustic potash on a slide, and looked at with a moderately high power (quarter inch) the so-called 'molluscum bodies' are seen—round cells at one time regarded as protozoa, but now known to be degenerated epithelial cells.

"The disease is very contagious, but the organism has not been identified. It is commoner in children and women, and I have in private practice seen two cases in women in which the disease was contracted in Turkish baths."

If we examine very briefly the pathological teaching here produced we find it consists essentially of two parts: firstly, that the molluscum bodies are epithelial cells; secondly, that they are not protozoa. Both propositions are false: the bodies are not epithelial cells, but something that grows inside

them ; this any third-year student could see if allowed to use his own vision to interpret nature. The second proposition is not based on the lecturer's own observations, but is mere repetition of the opinion of others. It has the same quality as hearsay evidence in a court of law, and it should be excluded from lectures with the same severity that is shown towards it in courts of law. The facile repetition of what is conceived to be the opinions of others by the *Magnæ Voces* of the medical profession has the effect of lulling to sleep the critical faculty of each generation of students, and when, as in this instance, it is the statement of an error, the ultimate effect may be as disastrous to the community as has been suppression of truth on the part of highly-placed politicians.

The average of belief in the profession is also to some extent based on what is written in special text-books. The "Cellular Pathology" of Virchow is still the sum of belief for the average practitioner in all that relates to tumours. The cell in this connection means a nucleated cell such as we see in normal tissues. In the first part of this work, published in 1903, in considering the cell as a biological unit, I wrote :—

> "There remain some forms in the earlier stages of which no nucleus can be demonstrated, and in them it is probable that the nuclear

matter is interfused with the cytoplasm, and that the differentiation of cytoplasm and nucleus takes place only as the animal approaches maturity."

Myxodictyum (Fig. 1) is an example of a

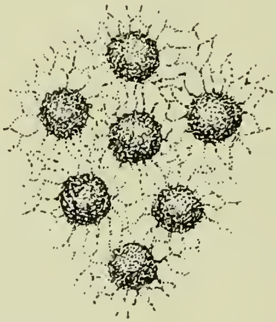


FIG. 1.—MYXODICTYUM: A COLONY OF NON-NUCLEATED PROTOZOA.

colony of non-nucleated protozoa. Of it Delage and Hérouard write:—

> "It is remarkable for the absence of a nucleus and for its pseudopodia, which form a rich network uniting it to neighbouring individuals, so that the animal forms colonies in which Haeckel has counted as many as seventy individuals. It thus approaches the condition characteristic of the Mycetozoa, with which, when its life-cycle is known, it may perhaps be classed."

As I show in Chapter IV. of the present

work, the non-nucleated chromidial individual is an important element in the life of at least one common mycetozoon. Before sending the MS. of this volume to the printer, I consulted the most recent English book on pathogenic protozoa, one published in 1914, and to see whether my knowledge was quite up to date I looked in the index for "chromidia," only to find the word absent. In the text I find in answer to the question, "What is a living unit?" the familiar picture of *Amœba proteus.* "This nucleus is most important," etc. For many years now we have known that chromidium-formation plays so important a part in the life of many protozoa that it is no more true to say of them that the nucleated cell is the biological unit than it would be to say that the three-layer blastoderm is the first stage of a man. Thus an impression is left on the reader's mind that is grossly misleading, and that on a subject which concerns the very foundation of biological and pathological knowledge.

I look again in the book I have referred to (an excellent book in many ways) in order to see whether my knowledge of the Mycetozoa is up to date. This is all I find:—

> "The Mycetozoa are a group of Protozoa, claimed equally by botanists and zoologists. . . . It is no part of the work of the present

> authors to attempt to decide the relative claims of zoologist and botanist, but we follow the lead of the authority on the subject, Dr. J. J. Lister, whose standing in the zoological world is beyond assail."

The authors do not even state what Dr. Lister's opinion of the mycetozoa is, or where it is recorded. These considerations are not merely academic, but they affect vitally the progress of medicine.

At the present time I do not see that the mind of the medical profession is better prepared for war with certain diseases than was the mind of the people of this Empire prepared for the frightfulness with which it is now in deadly combat.

CHAPTER II

NOTES ON A MYCETOZOON, AND A COMPARISON OF SOME OF ITS FORMS WITH ELEMENTS IN CYSTIC URETERITIS AND CANCER

In the temperate zone the mycetozoa are so wide-spread and abundant that theirs is no small place among the rich flora and fauna that flourish in dead vegetable matter, in and on which most of the many known varieties of mycetozoa live. One species[1] at one stage of its life feeds on the surface of living fungi. The known varieties are very numerous. A near congener to the mycetozoa[2] is familiar to us as the cause of a destructive disease in the cabbage and kindred food crops; others are here shown to cause disease in man.

The first mycetozoan genus to gain notice

[1] *Badhamia utricularis:* the plasmodium creeps on rotten bark and feeds on the surface of fungi growing therefrom, especially *Stereum hirsutum* and *Polyporus versicolor.*

[2] *Plasmodiophora brassicae*, Voronin, 1878. This parasite, which belongs to the Zoosporida, causes the tumours of the roots of the cabbage and other cruciferous plants, known popularly as "club-root," "finger and toe," or "anbury."

was Fuligo, more familiarly known under the name of the "Flowers of Tan," described in the year 1768.[1] On the surface of tan heaps or decaying wood its bright yellow growths may cover areas eight inches or more in width, and an inch or more in thickness.

The variety of form in the spore-containing organs (sporangia) of the different species, and in the other elements (capillitium, etc.) of the supporting framework, which these organisms secrete, and the fact that the capillitium has been found to give the chemical reactions of cellulose, naturally led botanists to claim as their own these organisms, which they named Myxomycetes, or slime-fungi ; but more critical study revealed the fact that at certain stages they form pseudopodia and incept and digest bacteria, and have other characters of the Rhizopoda or Sarcodina, the class of protozoa to which the amœbæ belong. Those workers who have studied them recently place them next to the highest rhizopods, and they are now generally known as Mycetozoa (fungus-animalcules), as de Bary named them in 1859.

A. Lister, whose monograph[2] is the foundation of English literature on the mycetozoa, quotes

[1] Haller, "Hist. Stirp. Helv.," iii. p. 110, 1768, is the reference given by A. Lister.

[2] "A Monograph of the Mycetozoa, being a Descriptive Catalogue of the Species in the Herbarium of the British Museum," by Arthur Lister, F.L.S., 1894.

Ray Lankester's opinion that they may be closely related to the Sporozoa. Calkins suggests that no harm can be done by including the mycetozoa in both the animal and vegetable kingdoms, for on *a priori* grounds it is to be expected that some organisms should be on the boundary line between artificial groups, such as the unicellular animals and plants. The term Protist is useful to designate organisms of doubtful position. In this book the mycetozoa will be considered as Rhizopod protozoa.

The first impression.—For years I had been trying to find some typical mycetozoon under circumstances that would permit me to watch its natural life, and to study it microscopically in its various phases.

It was not, however, till the spring of 1914 that I found the one here briefly described. A study of this protozoon, as can be gathered from the following notes, confirmed in a most signal manner the view of cancer that I have maintained for the past twenty-two years.

In a London garden, at its southern end against a boundary wall, is a raised border, shaded by apple and pear trees, and more closely by euonymus, box, holly, and other shrubs. Fallen leaves have been swept up and dug into the soil of this border in autumn. After a very wet and unusually warm

March, in the first days of April a number of whitish patches appeared on the surface of the soil. The patches varied in size, and looked as though a thickish gruel had been spilt, here a few drops, there a teaspoonful, there again as much as a tablespoonful; in some places enough to make a patch as large as the palm of a man's hand. The first patches appeared towards the west end of the border on which some sunshine falls on fine mornings. On the following days fresh patches of the growth were found towards the more shaded eastern part of the border. I put a portion of one of the patches with the earth it covered into a flower-pot saucer and took it into the house, having covered it with glass to keep it moist. The sample taken at 9 a.m. had changed its colour by midday to a yellow shade, looking like rich cream, and at points it had become heaped up into shining knobs, some as large as peas, some larger; the larger ones had secondary knobs smaller than pins' heads projecting from them. Meanwhile the surface of the patch, which was unbroken at first, had changed in such a way that small areas of the subjacent soil had been left uncovered, as shown in Fig. 2. When next examined about 6 p.m. the colour of the patch, except the yellow knobs, had changed to mauve. Next morning it was divided up into roundish purple segments, from 1 to 2 mm. in

diameter, of a colour so dark that at a little distance they could hardly be distinguished from the soil. The purple colour turned white when the growth was allowed to become quite dry.

Where a drop of rain remained in contact with the edge of a still growing patch after a shower

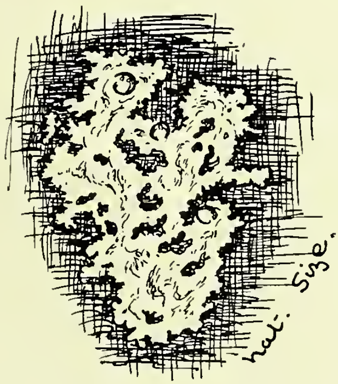


FIG. 2.—A PORTION OF ONE STAGE OF THE GROWTH.

a milky mixture formed. The different forms assumed by the growth are represented as if occurring on the same privet leaf (Fig. 3). During May and June fresh patches of growth continued to appear. One element, enlarged, is shown in Fig. 4. When detached from the leaf on which it grew the under-surface showed three layers: a middle purple, an outer thin white layer, and a central part, also white. After June no fresh growth was found throughout

the year. It was not until May 1st, 1915, that I next observed a small reappearance[1] at the edges of

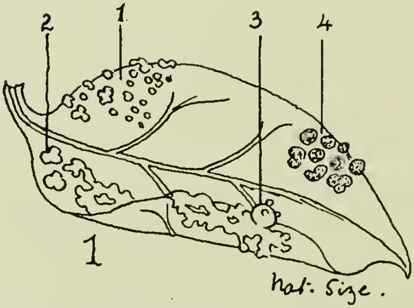


FIG. 3.—DIFFERENT VARIATIONS AND STAGES OF THE GROWTH, SHOWN AS IF ON THE SAME LEAF: (1) The smallest discrete elements; (2) larger discrete elements running together into a continuous patch, which at one point (3) is heaped up in a rounded nodule with small secondary projections; (4) spore-containing organs or sporangia.

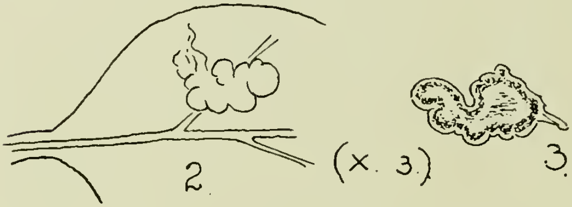


FIG. 4.—(2) A SPORANGIUM ON A PRIVET LEAF, ENLARGED; (3) THE SAME SPORANGIUM DETACHED AND VIEWED FROM UNDERNEATH.

a mound of leaves which had remained all winter at the east end of the border, where some sun falls in

[1] After this first reappearance, abundant growths appeared in all their varied modifications, and furnished me with the material

the afternoon. The winter had been one of the wettest, as soldiers in the trenches in Belgium found to their cost.

FIG. 5.—SPORES, CAPILLITIUM FILAMENTS, AND CRYSTALS. One of the spores has not yet formed its capsule, the beginning of which process I watched under the microscope. In its early stage the capsule is of a rose-colour, later the pigment is purple-brown and is condensed in the points which project from the surface of the capsule. Drawing eyepiece. × 800 diams.

---

which forms the basis of this chapter. The growths this year were limited to the leaf heap, no leaves having been dug into the earth last autumn. There was a transient exception to this: in two moist crevices I noticed a purplish bloom on the soil, and this the microscope showed to be due to a thin formation of spores with a few lime-crystals and some stunted, isolated structures which I took to be an abortive capillitium. Evidently Didymium requires a liberal diet. The growths on the leaf-heap ceased early in July, in spite of conditions that would seem to be ideal for hatching of the spores.

Microscopic Structure.—The purple elements, teased out and examined fresh under a 1-12th inch immersion lens, show the features drawn in Fig. 5. These are the spores, and cellulose filaments, or capillitium, of a typical mycetozoon, together with the lime crystals that occur in certain species.

Plasmodia.—The growth in the stage shown

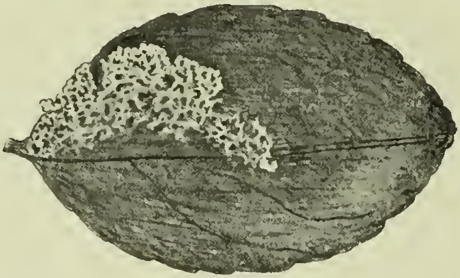


FIG. 6.—THE EARLY STAGE OF THE PLASMODIUM ON THE UNDER SURFACE OF A EUONYMUS LEAF. Natural size.

in Fig. 3; 1 and 2, is soft and easily broken up by needles. A portion of such a growth examined in water under a magnification of about 500 diameters is shown in Fig. 7. When first placed under the cover-glass there is in detached portions a lively oscillation of the granules, but this soon ceases.

These plasmodia are opaque and cream-yellow. Portions of them placed on a slide, and covered with a thin cover-glass, do not exhibit the

general rhythmic streaming which I have since observed in an earlier plasmodium from a culture referred to in Chapter III., p. 26.

After the publication of the preliminary note in which I briefly indicated the outlines of this study, I found among the leaves of the leaf-heap an

FIG. 7.—A PORTION OF THE EDGE OF A GROWING PLASMODIUM—*i.e.* COMPOSITE ANIMALCULE. There are numerous nuclei. One pear-shaped projection has become detached, and three smaller portions very like leucocytes. The nuclei are highly refracting, and have a greenish tint. Drawing eye-piece. × 500 diams.

earlier stage of the plasmodium. It was adherent to the *under* side of one Euonymus leaf, which was close pressed against another, and the adjacent surfaces of the leaves were wet. This plasmodium is sketched in Fig. 6. It was transparent, except at two points where the growth was thickened. In this early stage streaming would probably be present.

Last year I noted that some of the detached rounded portions were very like some of the larger phases of protozoa I have described in cancer, sarcoma, syphilis, small-pox, etc., but it was not until this present year, on examining a teased-out portion of one of the denser yellow knobs, which will be called "nodules" in the following account of them, under a 1-12th-inch lens that I beheld masses of bodies identical in form, internal structure, and optical properties with the larger of those bodies which I first described as protozoa in cancer in 1893.[1] These bodies are shown in Fig. 8. As in cancer, so in this mycetozoon these bodies exist in ponderable masses.

Not less striking to me was a subsequent observation made on teasing a nodule intermediate in naked-eye appearance between the yellow knobs and another kind of nodule which this mycetozoon forms: a translucent colourless nodule like a slightly opalescent glass bead and probably an early stage of the yellow nodule. On teasing a nodule translucent in its one half, and opaque and yellow in the other, I found the appearance shown in Fig. 9.

The globules shown in Fig. 9 resembled in

[1] "The Protozoa Considered in Relation to Disease." *Medical Press and Circular*, August 16th and 30th, September 6th, 20th, 27th, October 4th and 11th, 1893.

their chief physical characters some I found in the small cysts of the ureter in a case of protozoan

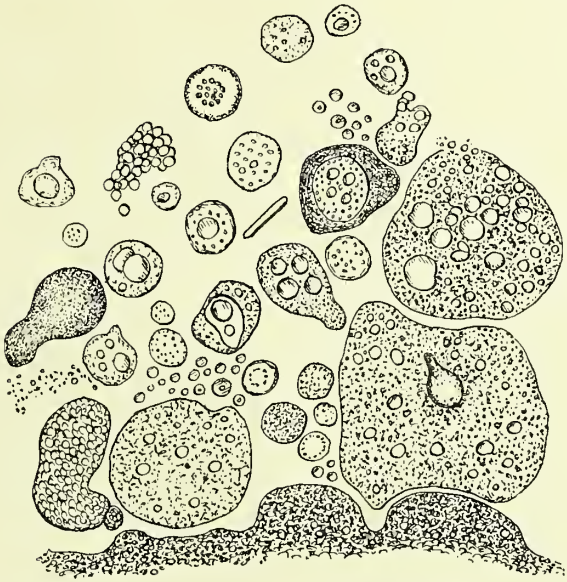


Fig. 8.—A Portion of One of the Rounded Yellow Nodules teased out in Water and examined under a 1-12-in. Lens. The various forms and the main mass indicated at the base were encased in a tough skin and constituted the bulk of the mass. There is neither capillitium nor spores. The nuclei have the same green tint and high refraction as those shown in Fig. 7. All these structures have their homologues in the most typical cancers and sarcomas. Drawing eye-piece. × 800 diams.

disease of the urinary tract in 1892.[1] The disease affected the left urinary tract of an elderly woman.

[1] "A Case of Peorospermial Cysts of the Kidney, etc." *Transactions of the Pathological Society of London*, 1892, p. 94.

In the fresh state the contents of the small cysts was of a buff colour, and under the microscope showed large and small, oval and irregular cells which contained bright globules. The contents of many

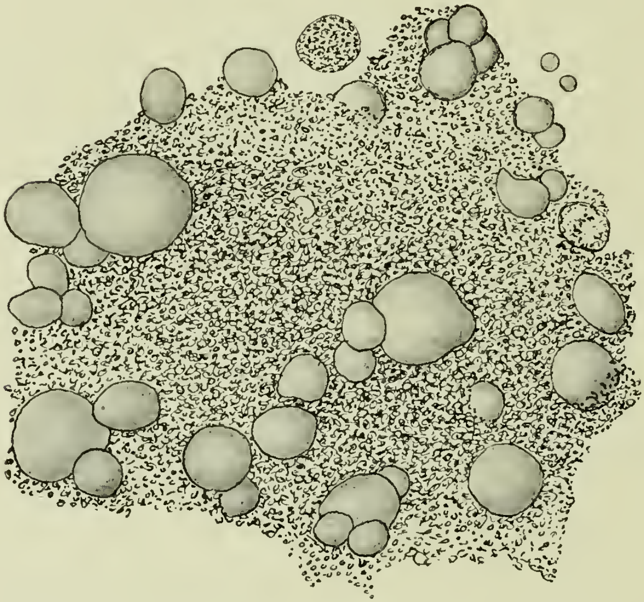


Fig. 9.—Part of a Teasing of a Mixed, Half Translucent, Half Opaque Nodule. The translucent spheroids were pink in colour and did not float up against the cover-glass as do drops of oil. Drawing eyepiece. × 800 diams.

of the cysts consisted of oval cells embedded in an albuminous material, others in place of the large oval nucleated cells contained small granular corpuscles and nucleated cells (see Figs. 20, 29, and 30).

The foregoing are the outlines of the subject as they first presented themselves to my eyes and mind, and it is incumbent on me to show how my observations are related to what is already established in biology concerning the objects concerned, and to give the basis of the bearing they appear to me to have upon the causes of certain diseases.

CHAPTER III

STUDY OF A MYCETOZOON—*continued*

THE STAGES OF THE MYCETOZOON EXAMINED IN THE LIVING STATE

THE mycetozoon here dealt with is doubtless *Didymium difforme*, which is thus described in A. Lister's monograph :—

> "*Didymium difforme*, Duby, Bot. Gall. ii. p. 858 (1830). Plasmodium colourless or pale yellow. Sporangia pulvinate on a broad base or irregularly elongated and forming plasmodiocarps, scattered, 0·4 to 2 mm. or more long, smooth white : sporangium—wall of two layers, the outer a thin crust of densely combined minute crystals of lime, separating from the iridescent inner layer, which is purplish or nearly colourless above, stout and yellowish-brown at the base, thickened at the margin. Columella none. Capillitium often very scanty, of coarse or delicate, purple or colourless, flattened threads, usually broad at the base, branching dichotomously and slender above. Spores dark purple-brown, faintly warted, 11 to 14 μ diam."

In the foregoing definition there is no mention of the parts of the growth which I have termed "nodules." The latter might, of course, be the result of some parasitic disease, such as a Chytridian infection, but this was disproved by careful microscopic examination, the results of which are detailed below.

Spores and zoospores.—In systematic classification the character and size of the spores are used as guides to the species, but variations occur. For instance, in *Didymium difforme* some of the spores are larger than others, and they are sometimes found to be linked together in pairs or chains by fusion of their capsules. Of the hatching of the zoospores or swarm-cell from the spore, A. Lister writes :—

> "The length of time that elapses before the germination of the spore after it has been placed in water varies with the species, sometimes and often in the same gathering of the species. *Didyminm difforme* produced abundant swarm-cells in twenty-eight hours, after three years and nine months from the date of collection, and in a few days all the spores appeared to have germinated, and plasmodia were formed in a moist chamber."[1]

[1] I regret that I have not been able to consult A Lister's "Cultivation of Mycetozoa from Spores," *Journal of the Linnean Society: Botany*, 1893, vol. 20, p. 529.

I must confess that the spores of the Mycetozoon have

A. Lister describes these processes as follows:—

> "The spore-wall is ruptured by the swelling of the contents, which slowly emerges through the opening, and in about ten minutes lies as a nearly pellucid globule by the side of the empty membrane; after remaining quiescent for a few minutes amœboid movements begin to take place, and shortly afterwards the flagellum is produced. . . . In about a quarter of an hour it acquires its full length of about 15 m., and by its lashing strokes the swarm-cell swims off with a dancing movement. . . .
>
> "The swarm-cell or zoospore is pear-shaped. The narrow end from which the flagellum arises contains the nucleus, which does not

proved somewhat grudging in showing me these developmental processes. Last year, kept on leaves moistened by tap water, and examined daily, they did not germinate: there were various different kinds of amœbæ among them, but these only fed on the spores. This year I allowed the spores to dry thoroughly for some weeks and then added rain water to them; this did cause a small percentage of the spores to rupture and allow their round crystalline spheres to escape. Typical amœbulæ I then found, but the great majority of the spores remained apparently unchanged. This hatching of the spores is not so simple a matter as some writers would seem to suggest. In some species the process is said to depend on the presence of certain bacteria.

Some similar difficulty in finding the exactly suitable surroundings explains my not having been able to repeat my single observation of flagellated zoospores in molluscum contagiosum as described in Chapter V of this book.

alter its position. The broad end is granular and the granules are in constant movement."

This protoplasmic motion in zoospores is comparable to that described below in molluscum bodies.

"In addition to the dancing motion, the swarm-cells when they come to rest exhibit

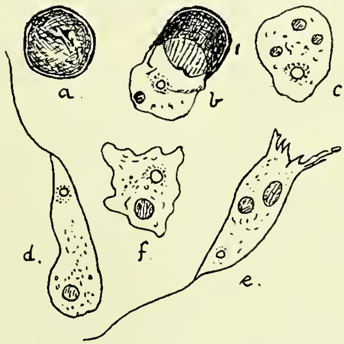


FIG. 10.—DIDYMIUM DIFFORME, DUBY. (*a*) Spore; (*b*) Swarm-cell escaping from spore-case; (*c*) Newly hatched swarm-cell containing a nucleus and three vacuoles; (*d*) Flagellated swarm-cell; (*e*) Swarm-cell with two vacuoles containing bacteria, and produced at the posterior end into pseudopodia, to one of which a bacterium is attached; (*f*) Amœboid swarm-cell. × 720 diams. After A. Lister.

movements of an amœboid character, and spread with an irregular outline; or they assume a linear form and creep over a level surface with a snail-like motion, the flagellum being extended in advance. In this position the movement of the interior substance is seen to advantage. In the large swarm-cells of *Amaurochaete atra* it may almost be described

as streaming, the granules passing from one end to the other in constant flow ; the hyaloplasmic extension at the posterior end continually changes its form, and often detaches portions which cannot be distinguished from the rest of the hyaline element, and appear to contain refuse matter. After a time the creeping is again exchanged for the dancing movement."

In all cultivations of germinating spores, a number of the swarm-cells, after a short period of activity, withdraw the flagellum and become encysted in a globular form, as the microcysts of Cienowsky. After being dried and re-wetted, the animalcule bursts the membranous cyst-wall and emerges to resume the swarm-cell form.

The plasmodium or creeping film. — Once formed the plasmodium moves like a huge amœba. When first formed it is transparent and colourless, and a portion can easily be detached by needles and placed in a drop of water on a slide, covered with a very thin cover-glass and examined. If the preparation is prevented from drying the vital phenomena continue for hours. In Fig. 11 is shown a part of the edge of a plasmodium with its pseudopodia. By means of arrows, the direction of the outward current is indicated. The latter continued over a minute and was then reversed.

The preparation just referred to was obtained from the most successful culture I have as yet made. It was arranged as follows :—

A leaf bearing some sporangia and a dried plasmodium (sclerotium) were put in a glass dish together with some partially exhausted tea-leaves

Fig. 11.—Pseudopodia at the Margin of a Young Plasmodium grown in a Culture. The arrows show the course of the stream of finer granules, the coarser granules only are shown in the figure: they remained stationary in groups. × 800 diams.

and sufficient tap-water to reach the lowest parts of the leaves when the vessel was inclined, as shown in Fig. 12. The water was kept as nearly as possible at the same level by pouring from time to time a few drops of tap-water upon the uppermost portion of the little pile of tea-leaves.

Such being the events at the advancing margin of the plasmodium, we may pass to consider

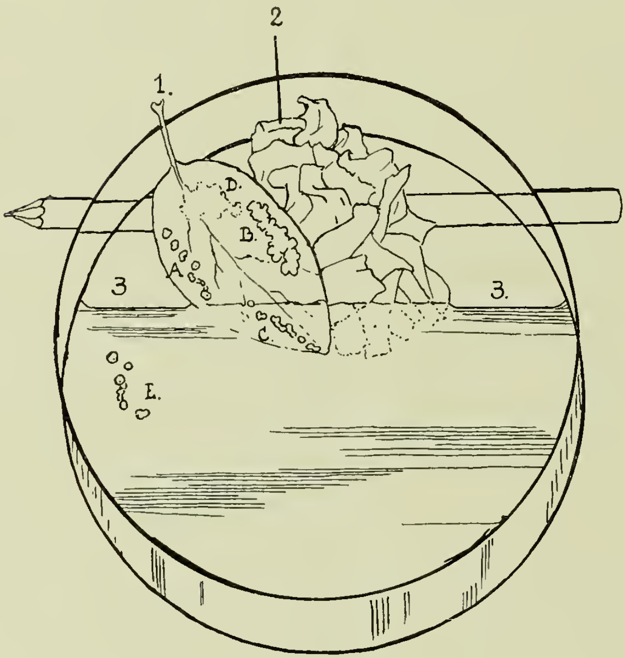


Fig. 12.—A Flat Culture-Dish slightly tilted by placing a Pencil under it. 1. A leaf, which had on it when first placed in the dish the group of sporangia, A, and the sclerotium, B; 2. A little heap of tea-leaves; 3 The level of the water. The preparation was made on the 9th of June; on the 3rd of July there appeared: C, a group of sporangia, and, D, a transparent plasmodium; on the surface of the water, E, another group of sporangia, which is drawn on a larger scale in Fig. 16, p. 34.

the protoplasmic currents in the main mass. A. Lister has described them as follows:—

"The movements in the interior of the swarm-cell are extended into a system of circulation in the plasmodium, which spreads in a network of veins with a few principal channels. Through these the granular substance streams in a rapid torrent, which gradually comes to a pause in the space of a minute and a half to two minutes; it then immediately reverses its course, maintaining a rhythmic flow, backwards and forwards at nearly equal intervals, but always of a somewhat longer duration in the direction in which the plasmodium is creeping."

A. Lister compares the movement of the plasmodial plasm to that of the zoospores.

A portion of a sclerotial part of the growth that had been in a moist chamber for a few days teased out in water, and examined under a 1-12th-inch immersion lens showed an active advancing edge, but no streaming movement. The method of advance was as follows: a row of hyaline pseudopodia were pushed out simultaneously, Fig. 13, 1. In the course of about a minute, the pseudopodia had become granular by the extension of the granules of the deeper part of the plasmodium extending into them, and partly by their own retraction. This process I watched in detail in a pseudopodium (Fig. 13, 2), which was rather more prominent and watery

than those adjoining it. The balloon-shaped protru-

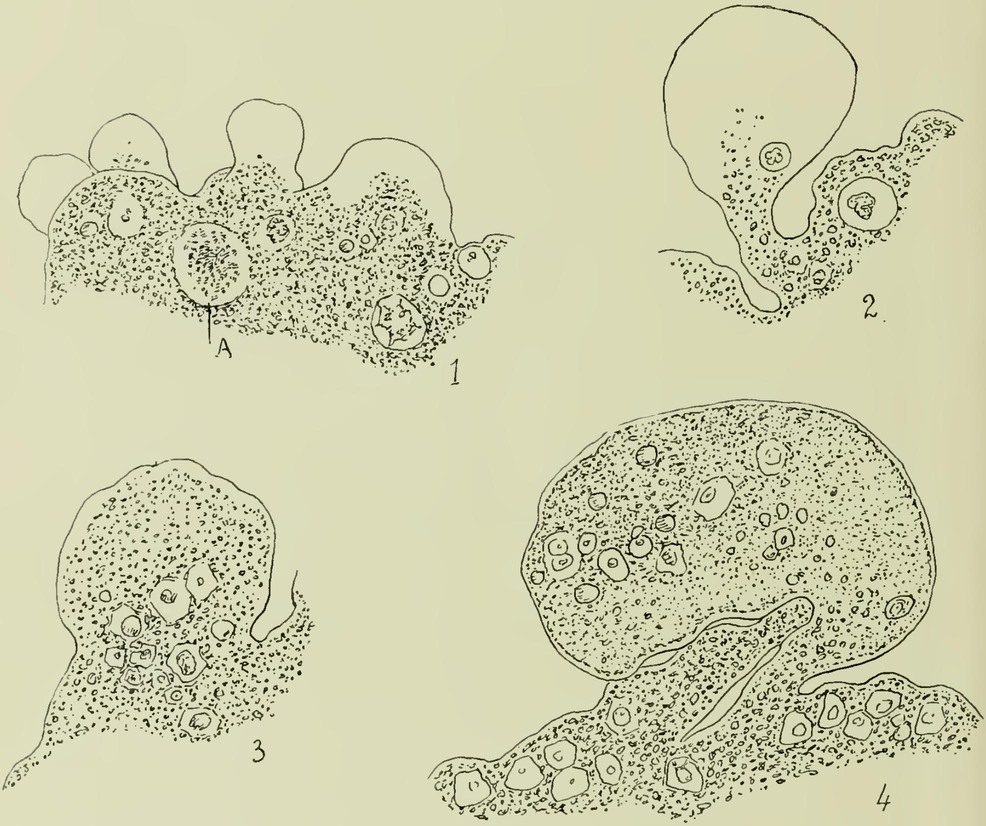


FIG. 13.—THE GROWING EDGE OF A PLASMODIUM. 1. Four pseudopodia, A, a vacuole containing bacteria; 2. A balloon-shaped pseudopodium into which granular plasm is streaming; 3. The same as 2 about a minute later; 4. The same as 3 after another minute. × 800 diams.

sion was at first devoid of granules; these advanced into it through its attaching stalk, and when they

reached the dilated part they began to oscillate. The influx of granular matter continued till the pseudopodium was filled (Fig. 13, 3). It even went further, causing the protrusion to become more than double its original size, and to fall over against the main mass of the plasmodium (Fig. 13, 4), appearing to fuse with the latter, and it contracted in volume appreciably as it did so. The pseudopodium had thus become part of the mass of the plasmodium.

During these processes the nuclei in the pseudopodium increased in number, and in the case of one of them I watched its formation: in a clear part two granules were seen close together, other granules joined them and all fused together, forming the highest nucleus (Fig. 13, 4). Doubtless some of the nuclei were brought by the inflowing endoplasm, and I think they had been formed in the same way: by fusion of chromidial granules.

Development of spores from plasmodia.—On searching teasings of sporangia to trace the origin of the spores and any nuclear changes that may occur in this process, I have not been able to detect any mitotic figures in the fresh living plasmodium. The nuclei appear to be double in some of the spores, and where two nuclei are present they appear to differ in character, Fig. 14 (*a*). In one instance I observed

such a double nucleus change into a single one by fusion.

In the plasmodium nuclear fusions also occur, as will be more fully considered in Chapter IV.

In the examination of plasmodia that have

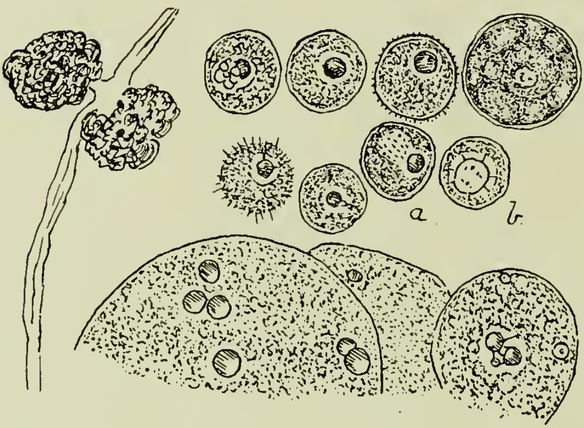


FIG. 14.—SPORE-FORMATION, FROM A TEASING. Part of the plasmodium is seen below, the lobe on the right showing a compound nucleus. To the left a capillitium fibre with two pigmented masses. There are a number of young spores, of which one is larger than the others. Some of the nuclei of the spores are double; that in (*a*) was observed to change to the same type as that in (*b*). × 800 diams.

developed from old sclerotia, care must be taken not to confuse forms of Chytridian parasites with those of the mycetozoon, see Fig. 15. These parasites are dealt with in Chapter XII.

Sporangia.—As mentioned in Chapter II., the formation of spore-containing organs or sporangia

from the plasmodium is fairly rapid, taking place in the course of a few hours. The determining agency cannot be want of water, for I have observed sporangia form in a culture on the surface of water

Fig. 15.—A Part of a Teasing showing below coarsely Granular Undifferentiated Substance. Above this is a Group of Spherical Bodies, the Early Stage of Spores. (*a*) Is a chytridian cell, in which a septum is forming; (*b*) A much elongated chytridian rod with several septa; (*c*) A chytridian close to a spore of the mycetozoon; (*d, e*) Spindle-shaped chytridian cells, one with nucleus and vacuole. × 800 diams.

thinly covered by a film of bacteria, etc., stained with tannin from tea-leaves used as nutriment (Fig. 16).

When first observed these sporangia were of a light lemon colour. In the course of about two hours they changed to lilac, and soon after they had become dark purple. The two more typical ones, Fig. 16 (*a*) and (*b*), have small pointed wing-like

projections at their base; continuous with (*b*) and close to (*a*) are sclerotial-like structures; in (*d*) the sclerotial part is as large as the sporangial part. In (*c*) the fructification has taken place without the plasmodial form being changed, constituting a plasmodiocarp.

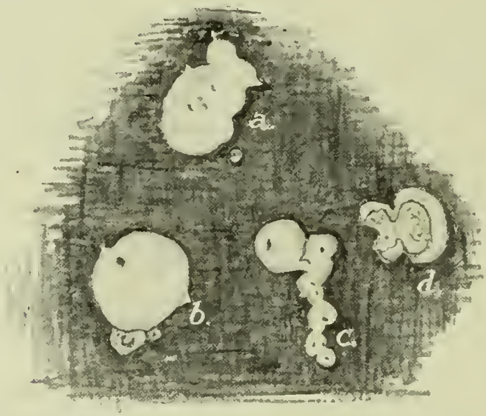


FIG. 16.—FOUR SPORANGIA FROM THE CULTURE DISH. × about 10 diams. Details in text.

The sclerotium.—A. Lister writes:—

"Superficial plasmodia may pass into the resting stage or sclerotium, and this change may be induced by exposure to dry air. In some cases, however, it occurs when water and apparently food material are present, and the cause for the change is then difficult to discover. When the plasmodium of *Badhamia utricularis* is dried, the streaming movement

gradually ceases, and the granular particles collect in clusters, surrounded by a border of hyaloplasm; the refuse matter is thrown out, a membranous cyst-wall forms round each cluster of granules, which also includes ten to twenty nuclei; the cysts become agglomerated into thick masses of irregular shape, drying to a horny consistence."

The framework.—Under this head I would include not only the capillitium and in those species that have it, the stalk or hypothallus, but also the protecting membranes, and the various kinds of capsules, including those of the spores. All are formed by a modification of hyaloplasm as can be followed under the microscope. This subject will be examined further below.

The formation of a sporangium has been graphically described by A. Lister:—

"In examining the rising sporangia of *Physarum nutans* in a moist chamber under the microscope, the projecting masses of plasmodium are seen to pulsate, distending and shrinking as the rhythmic flow advances and retreats, but gradually gaining with the advancing movement. The basal part of each contracts and forms a stalk consisting of a tube of tougher hyaline substance through which the protoplasm continues to pass until

the surrounding veins have emptied their contents into the spherical sporangium "

Lister's observations also show that the lime crystals can be dissolved by the plasmodium and re-secreted to appear either in the nodes of the capillitium or in the covering membrane of the sporangium.

FIG. 17.—A PART OF THE SURFACE OF A FIRM TRANSPARENT NODULE TEASED OUT IN WATER. Note the fewness of nuclei, and the brittle cleavage at the torn edge. Drawing eye-piece. × 600 diams.

The nodules.—This term I apply to the rounded knob-like protrusions from the plasmodium; they vary in size from that of a lentil to that of a split-pea, and they are of three kinds:—

1. Translucent and colourless, like a drop of boiled starch that has set; this type of nodule is soft and yet brittle.

2. Opaque yellow, looking like a drop of inspissated drab paint, and having sometimes minute secondary protrusions.

3. Intermediate form, partly translucent, partly opaque.

The appearances seen when portions of the living nodules are teased out in water are shown in Figs. 8, 9, 17, and Plate II.

Whether every nodule passes through the translucent stage or not, I am not sure, but that some do so is evidenced by the occurrence of intermediate stages. It is possible that the "milky-mixture" referred to above as being formed where a drop of rain-water remains in contact with a plasmodium (see Plate II., Fig. *c*) may become a translucent nodule; if so, it would be an instance of an animalcule having the power to dilute itself with water, a point which may have an important bearing on filterable viruses of disease.

Other details of teasings of a yellow nodule, and the appearance of a drop of the "milky mixture" already referred to, are shown in Plate II.

Large encapsuled cells.—These are four times the diameter of an average spore, and may be contained in pairs in a common capsule (see Fig. 18, B, C). They are obtained by teasing portions of sclerotium that have been kept wet for seven days

or longer. They exhibit phenomena so astonishing to me that I should hesitate to mention them had I not observed them carefully on several occasions, and for hours together. Fig. 18, A, shows a single large cell with a coarsely recticular nucleus and no nucleolus. The initial stage of such a nucleus is shown from another similar cell. I watched the nucleus first as a uniform very finely granular sphere connected with the cytoplasm by radiating rows of granules; then it became separated from the latter by an even space, as shown in Fig. 18, *a*. The homogeneous nucleus then changed to one of highly refracting granules. In this stage they resembled the upper of the two cells in Fig. 20. Later the granules are contained within an oval membrane and surrounded by nuclear juice. The large forms encapsuled in pairs (Fig. 18, B, C) were each enclosed in a doubly contured capsule, which in the drawings is represented for sake of simplicity by a single outline. The space between the outer capsule and the contained cells was occupied by coarse granules, which, like the capsules, were of a brown colour. The cytoplasm of the cells, a detail omitted for clearness, was also granular, but more finely so than that of the outer space. The nuclei of both cells were amœboid, being now of definite oval form, now sending out processes of its network into the cytoplasm; the latter changed its

PLATE II

TEASING OF A YELLOW NODULE. (*a*) Shows at the lower part a number of oil drops; (*b*) The appearance observed in the same preparation as (*a*) on being left to dry: this shows the beginning of the sclerotium stage; (*c*) Elements in the "milky mixture" formed by the edge of a plasmodium mixing with a drop of rain at its edge; (*d*) An oval non-nucleated body from a yellow nodule; (*e*) Part of a teasing showing the independence of the nuclei from the plasm; (*f*) rod-like bodies, one of which shows some differentiation of interior. × 800 diams.

appearance, becoming more densely granular, or showing centripetal striæ where the two cells abutted

FIG. 18.—A, A large encapsuled cell with a coarse reticular nucleus; (*a*) An earlier stage of a nucleus similar to that in A; B, A pair of large encapsuled cells enclosed in a common capsule; on each side is shown the optical section of a zone of coarse granules lying between the capsules of the cells and the common capsule: the outlines of the nuclei and of the epurating vesicles, the arrows show the direction of movement of the latter; (*b*) The beginning of a septum which was seen to grow across the cell; C, A pair similar to B, but with the nuclear reticulum branching into the cytoplasm in the upper cell, and radial striæ around the periphery of the cytoplasms and the vesicles; (*c*) the nucleus of the upper cell in C in another phase, sending out a pseudopodium towards the vesicle. Drawing eyepiece. × 800 diams.

on one another, and sometimes at the whole periphery. The most remarkable feature of all consisted in the

presence of a bright globule, or sometimes two such, which made regular journeys from the median border of each nucleus to the side where the coarse granules lay under the common capsule at the left side, always to the same spot. Each double journey occupied about two minutes. The globules probably are epurating organs, a special form of contractile vacuole, but it appeared to me that they also subserve a nutriment-carrying function. On one occasion, as indicated in Fig. 18, *c*, the globule on reaching the end of its inward journey was met by a suddenly pushed-out pseudopodium from the nucleus of one of the cells, and the whole globule seemed to be engulphed by the nucleus. This event was followed by a dancing motion in the cytoplasmic granules of both cells; this motion lasted some minutes. The encapsulation of cells in pairs recalls the similar occurrence in gregarines, but in this mycetozoon each cell of the pair is separately encapsuled. What I believe to be the beginning of encapsulation is shown in Fig. 18, *b*. The oval coarsely granular cell was enclosed in a capsule of a pink colour, like that of the early stage of the capsule of the ordinary spore. A branch of the capsule is seen partly dividing the mass into two. As I watched this branch it increased in length until it joined the opposite margin, completing the division.

Comparison with cancer, etc.—Cells with radial arrangement of cytoplasm, peripheral or perinuclear, are common in cancer and sarcoma A giant nucleus sending processes of its network into the cytoplasm is shown in Fig. 19.

It was on finding that some of the oval bodies of cystic ureteritis had well-developed nuclei (Fig. 20), that I concluded that they could not be degenerated cells. The different stages of these nuclei of the protozoa of cystic ureteritis closely resemble those described above in *Dydimium difforme.*

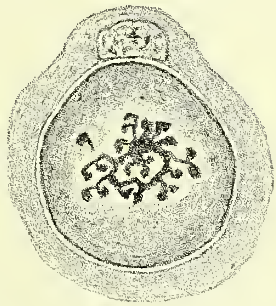


FIG. 19.—CANCER. An intracellular parasite which I described in 1893 as showing a "giant mitosis"; I now think it more probably an intracellular mycetozoon with an amœboid nucleus similar to that shown in Fig. 18, c.

Rod-shaped elements.—In teasings the rod-like forms that have been referred to above are frequently met with. They occur in the large yellow nodules. The most abundant formation of them that I have come across was in teasing a small sclerotial brownish-yellow point of growth that appeared with other forms in a culture on tea-leaves. The cluster of rod-like bodies is shown in Fig. 21. They reminded me strongly of the microgametes of a Klossia

I saw in the kidneys of small slugs, 1885.[1] They separate from a non-nucleated hyaline, *i.e.* ground-glass like substance which has the same optical characters as molluscum bodies, and many bodies of cancer and sarcoma.

Fig. 20.—Two Cells from a Cyst in Cystic Ureteritis. The upper has a nucleus similar to the middle stage of nucleus formation in the large cells described in Fig. 18. × about 800 diams. From the *Transactions of the Pathological Society of London*, 1892.

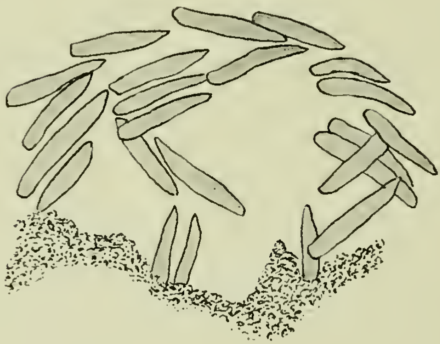


Fig. 21.—A Number of Rods as seen in a Teasing. The lower ones are in contact with the finely granular highly refracting substance from which they are probably formed. × 800 diams.

At first dense and uniform in texture they sometimes become granular internally. They are to be distinguished from the phases of Chytridians as shown in Fig. 15. Next year I hope to be able to study these rod-shaped elements more completely.

[1] J. J. C., "Notes on Various Sporozoa," *Q.J.M.S.* 1895.

CHAPTER IV

DIDYMIUM DIFFORME—THE MINUTE STRUCTURE AS SEEN IN FIXED AND STAINED PREPARATIONS

This part of the subject will be best introduced by stating in outline what I have been able to glean of the established biological view of this important aspect of Mycetozoa, and then to detail my own observations. With regard to the latter it may be stated in advance that chromidium formation greatly predominates over the more familiar nuclear processes. I have the pleasure of thanking Dr. H. W. Perkins, Pathologist to the Hampstead and N.W. London Hospital, very warmly for ungrudging help in the preparation of sections for me. At my request the stain used was acid hæmatoxylin with eosin as ground stain, this being the stain commonly used for ordinary pathological diagnosis.

Nuclear Processes.—The appearance of the zoospore when fixed and stained and its mode of division are shown in Fig. 22. A. Lister[1] described

[1] Arthur Lister, "A Monograph of the Mycetozoa," 1894, p. 4.

division of the flagellated zoospores by karyokinesis with minute spindles (see Fig. 22), and on one

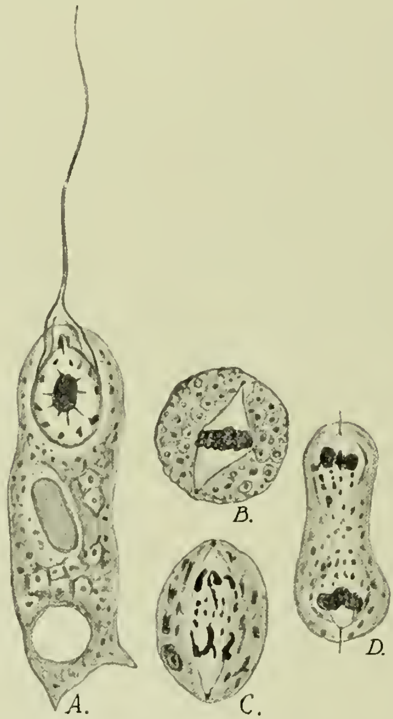


FIG. 22.—THE ZOOSPORES OF STEMONITIS FLACCIDA (LISTER) FIXED AND STAINED. A, The fully developed myxoflagellate showing the relation of the flagellum to the nucleus; a contractile and a food vacuole are present; B–D, Stages in mitotic division; D shows the formation of the flagellum from the centrosome. From Doflein after Jahn.

occasion found similar spindles in one part of the creeping film of *Badhamia utricularis*, but on farther investigation found that could not be the only way by

which the nuclei increase in number. The plasmodium of this mycetozoon is capable of increasing in size sixfold in twenty hours, and Lister found in fifty-five smears on cover-slips made at intervals of fifteen minutes that the nuclei increased in numbers *pari passu* with the growth of the plasmodium. No spindles were found, and the observer concluded that they increased by direct division. The important chromidial condition of protozoa was at that date but little known. Lister's drawing of the nuclei of Badhamia leads me to believe that the nuclei there seen are similar in type to those in Figs. 25 and 26, and I have little doubt that they were produced in the same way, that is by concentration of chromidial granules, and subsequent differentiation.

The earlier view of the nuclear changes preceding spore-formation was that of Jahn and Kränzlein, to the effect that fusion of nuclei took place in pairs throughout the plasmodium, and this was followed by reducing divisions. The later view is stated as follows: nuclear fusions take place only between degenerating nuclei, and are vegetative, not sexual. The true sexual process begins with the nuclear division which precedes spore-formation: it is a true reducing division, the chromosomes being reduced from 16 to 8. The nuclei of the spores and of the amœbulæ and zoospoores have half the full

number of chromosomes. In *Physarum didermoides* the amœbulæ multiply by repeated fission with mitoses showing 8 chromosomes, then couple in pairs; the zygotes form the plasmodium. When a young zygote meets an amœbula, it ingests and digests it.[1] The nuclei in the plasmodium multiply by fission with 16 chromosomes.

Fig. 23.—Section of Part of the Plasmodium, Early Stage, with the Leaf on which it grew: that shown in Fig. 6, Chap. II. It is seen that there is no intimate connection between the mycetozoon and the leaf. Drawing eye-piece. × 100 diams.

Plasmodium, early stage.—I will begin my personal observations with the early plasmodium shown in Fig. 6, Chap. II. A section of a strand of the plasmodium, with the portion of leaf to which it adhered, is shown in Fig. 23 as seen under a low

[1] This is not the only kind of cannibalism Mycetozoa are charged with: the young plasmodium is said to ingest and digest any microcysts within its reach.

power. There is no intimate connection between the plasmodium and the leaf. The plasmodium stained with hæmatoxylin and eosin is of a blue tint in every part.

Under a high power the plasmodium is seen (Fig. 24) to present a very fine alveolar structure throughout. There are no nuclei. Certain areas stained rather more deeply probably represent the sites

Fig. 24.—Didymium Difforme. Part of the section of the plasmodium shown in Fig. 23. It is a fine alveolar non-nucleated plasmodium in the chromidial condition. Drawing eye-piece. × 800 diams.

of nuclei which have undergone complete solution into chromidial fluid, the chromatic particles of which are too small to be individually recognised. At this stage the plasmodium is in the chromidial state in every part.

I am sure that any Virchowian pathologist looking at the structure shown in Fig. 24 would pass it by as degenerated material, yet it shows the structure of a compound protozoon in a state of

perfect vitality. The specimen proves that Nature has not constructed the protozoa in harmony with Virchow's views.

Plasmodium, second stage.—In sections of the yellow plasmodium, that is at the stage at which spores are about to form, the multi-nucleate, folded plasmodium stains a reddish-purple colour, contrasting with the blue tint of the early stage just described. But amongst these folds of the forming sporangium portions of plasmodium of a different aspect are to be found. These are stained a blue-purple like the early plasmodium. They are traversed by regular parallel fissures. Under a high power (Fig. 25) the nuclei are seen to be stellate, and they can be traced as being formed by coalescence of chromidial particles. The general basis of the plasmodium looks chiefly alveolar in some lights, in other lights chiefly granular: in reality it is alveolar with condensations at the nodal points, as is seen where its elements are more loosely arranged (Fig. 25, a). I have no doubt that this is a stage intermediate between those shown in Figs. 24 and 26.

Plasmodium, third stage.—The varied detail of structure seen in a section of a plasmodium ripe for spore-formation, as seen in the frontispiece, Figs. 1 and 2, almost justify the application of the term "histology" to the account of its structure.

The appearance of part of a plasmodium in this late stage, as seen under a lower power, Frontispiece, Fig. 1, is oddly reminiscent of a gland

a

b

Fig. 25.—The Intermediate Stage of the Plasmodium. There are numerous stellate nuclei. Where the structure is loose, as at (a), the plasmodium is seen to be composed of stellate elements linked together; the supporting framework (b) is seen to be forming by transformation of the outer layers of the plasmodium. Drawing eye-piece. × 800 diams.

such as a suprarenal; but a closer view shows a want of definite cell-outlines, and most of the nuclei are too small to be those of any human gland.[1]

[1] The view that all the cells of cancer are parasites, as expressed by Adamkiewicz, L. Pfeiffer, and the late H. T. Butlin

A part of the same pre-sporangial stage as is shown in Frontispiece, Fig. 1, revealed the very interesting picture given in the same plate, Fig 2, when placed under the oil-immersion lens: the presence of numbers of nuclei round the clefts and spaces in the equatorial stage of mitosis. Careful search failed to show a nucleus in any other stage of mitosis. If this is, as I suppose, the nuclear division preceding spore formation, it does not in Didymium altogether bear out what has been given in the early part of this section as the accepted view of the process of sporulation; this will be better seen when the next stage is examined.

The sporing process.—Another part of the same plasmodium, the Frontispiece, Figs. 1 and 2, examined under a high power (Fig. 26) shows part of the framework, like a basement membrane, to be continuous with a network, which again is continuous with a filament of the capillitium. In the meshes of the net is a round body densely stained with hæmatoxylin, probably an early stage of a spore. The

among others, has never been held by the writer. The reticulum shown in Fig. 27 could not be fibrin, which is much more delicate in structure. I may also mention that before the stage of the protozoa of cancer represented in Fig. 27 is reached (I called it the sporing stage in 1893), numerous mitotic points appear in some instances, and they may be homologous with the minute mitoses shown in Frontispiece, Fig. 2.

basis of the growth is of fine foam-like protoplasm, between the globules of which lie minute granules staining like chromatin. At one point these granules

FIG. 26.—A PART OF A THICK PLASMODIUM OR EARLY SPORANGIUM. To the left one of the large chromidial masses has absorbed one nucleus and is in process of absorbing another. The supporting framework contains a spore. The upper right portion of the figure was introduced from another part of the same section and given a similar connection with the framework to show the continuity of the capillitium with the framework. Drawing eye-piece. × 800 diams.

can be seen to be continuous, with a densely stained spherical mass. Another similar mass is seen to have absorbed one nucleus, and to be in process of absorbing another. The meaning of these points:

the minute mitoses may be a stage of reduction, but they are too small for me to count the chromosomes with the magnification of 800 diams., and as certainly they do not appear to be followed immediately by subdivision of the mass into spores, but there ensues a formation of highly chromatic "pools," I venture to name them, since they result from a pooling of chromidial assets of all kinds: granules from the plasmodium, and nuclei of various values. It appears to me that some of these spherical pools become converted into the spores. They also play a part in the formation of some portion of the capillitium. I hope to be able to examine and describe them more extensively at some future time.

Comparison may be made between the sporing mycetozoon and one of the elements which I described as protozoa in a cancer of the septum of the nose in 1892. This structure is shown in Fig. 27, from a hæmatoxylin stained section. There is a framework continuous with a sac containing six leucocytes and a number of bodies which are stained faintly with hæmatoxylin throughout.

Examination of the nodules in sections.—The yellow nodule affords most important data. Some of these are shown, Frontispiece, Fig. 3, as seen under a low power. Note there the central hyaline mass, which takes the hæmatoxylin and the eosin of the

stain equally. Apart from some wavy lines of deeper stain, some slight vacuolation, and near the lower edge some nuclei, there is no recognisable differentiation of structure. Note also that the lower

Fig. 27.—A Structure from a Squamous-celled Cancer: a Sac, the Wall of which is continuous with a Reticular Framework like that shown in the Mycetozoon in Fig. 26. The sac contains six leucocytes and a number of reticular bodies which stain with hæmatoxylin throughout. In the meshes of the framework are four bodies of hyaline appearance, stained with hæmatoxylin: these are stages of the parasites previous to that of sporing. Drawing eye-piece. × 800 diams.

limit of this drawing represents a cleavage in the section, suggesting a horny texture in the hardened material. There are other hyaline masses present, and at their margins and between them a less

continuous and less deeply stained substance thickly dotted with dark points—nuclei as they are shown to be under a higher power (Fig. 28).

Now let us compare this section of Didymium with a section and a smear-preparation of protozoan

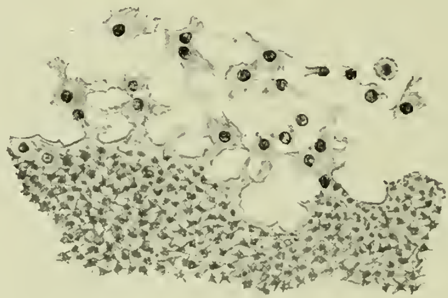


FIG. 28.—DIDYMIUM: A YELLOW NODULE. A portion of the margin of the central hyaline mass above (*a*) in Frontispiece. The hyaline substance is seen to consist of minute stellate subdivisions; some of the stellate subdivisions have become rounded and are passing outwards to form the nucleus of one of the leucocyte-like amœbæ. The nuclei of these newly formed amœbæ are of chromatin. The amœbulæ exactly resemble small lymphocytes in human tissues. Drawing eye-piece. × 800 diams.

cystic ureteritis as described in Part II. of this work. Fig. 29 shows six cysts with their contents; the latter correspond in every particular to the contents of the yellow nodule of the Mycetozoon. The smear also (Fig. 30) shows a hyaline substance, the semi-fluid nature of which in the fresh state allowed it to spread in an even layer on the slide. Some of the amœbæ

in cystic ureteritis have densely chromatic nuclei like those of the Mycetozoon, whilst the denser parts of the others still keep to the chromidial type. These resemblances are too close to be merely chance pheno-

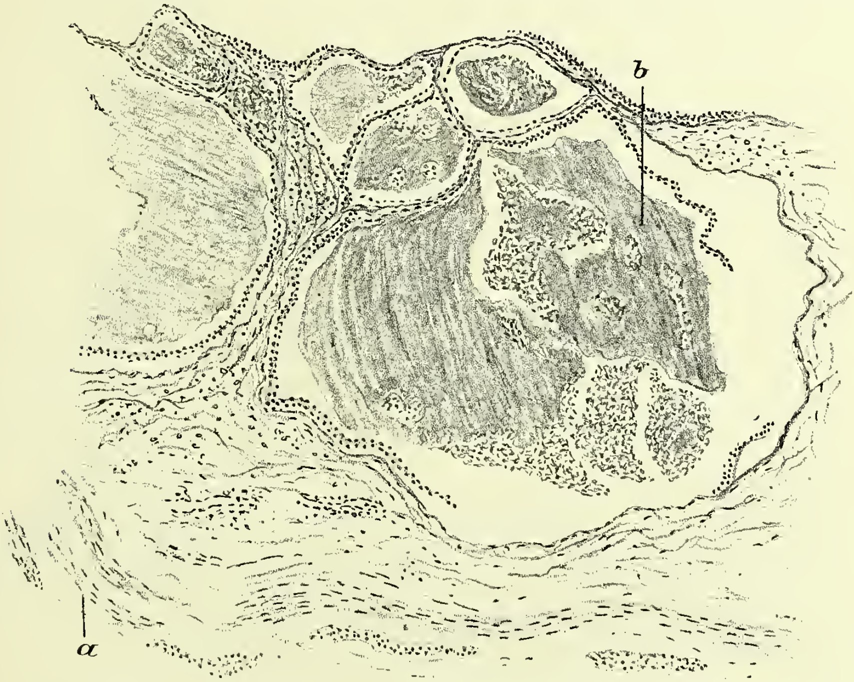


Fig. 29.—Cysts from a Case of Protozoan Cystic Ureteritis. Showing hyaline chromidium being transformed into amœba-like subdivisions. × about 100 diams. From Part II. of this work, published in 1908.

mena: the Mycetozoon and the protozoa of cystic disease of the urinary tract are evidently near akin.

In the section of which a part is drawn in Frontispiece, Fig. 3, it may be noticed that there are

present some roundish masses, in parts more deeply stained than the larger hyaline masses. Under a higher power they are seen to be identical with bodies that occur in rapidly growing cancers and sarcomas (Fig. 31).

The fusion of chromidial particles and small

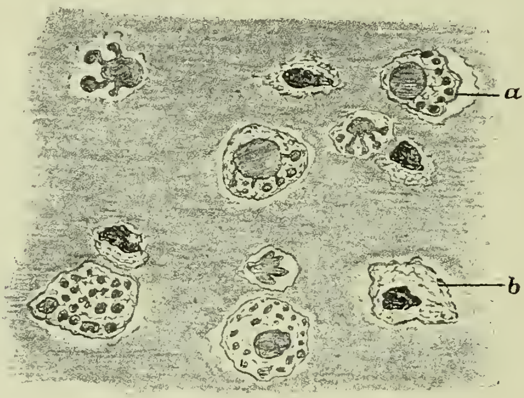


FIG. 30.—CYSTIC DISEASE OF THE URINARY TRACT. Part of a cover-glass preparation of the contents of a cyst stained with acid hæmatoxylin: *a*, Amœbæ, partly dense and partly reticular, no chromatin present; *b*, Similar body, but with dense chromic nucleus. Drawing eye-piece. × 800 diams. From Part II. of this work.

nuclei constituting chromidial pools is seen most strikingly in the partly translucent, partly opaque nodules (Fig. 32). These larger nuclear masses in the fresh state resemble some of the bodies of cystic ureteritis; they probably fuse together to form hyaline masses such as those shown in the Frontispiece, Fig. 3.

W. Ford Robertson, in collaboration with H. Wade, has made many very interesting observations in reference to mycetozoa and certain diseases of man and animals, namely, cancer and sarcoma, and cancer of mice, and infective sarcoma of dogs: three of which diseases I had previously described as

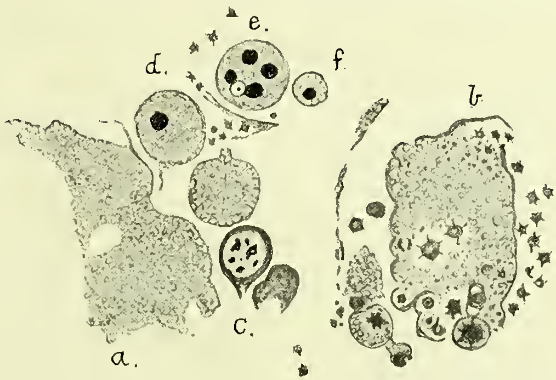


Fig. 31.—Didymium. Part of a stained section of a yellow nodule showing forms similar to bodies that occur in typical cancers and sarcomas.[1] *a*, Undifferentiated substance; *b*, A portion subdividing into amœbulæ; *c*, *d*, *e*, Bodies containing differentiated chromatin; *f*, A body resembling a mononuclear leucocyte. Drawing eye-piece. × 800 diams.

being caused by protozoa. It is difficult to do justice to this work because, from the limitations of time, etc., the authors do not show how their results are related to existing biology and pathology. To some

[1] The optical characters, high refraction, and the peculiar foam-like appearance of the more transparent parts of the bodies, shown in Fig. 31, are exactly repeated in the similar structures in cancer and sarcoma.

extent Dr. Ford Robertson has remedied this defect in a private letter to myself, dated December 3, 1909. It may be explained that their views are based on complicated cytological methods, a fact which will

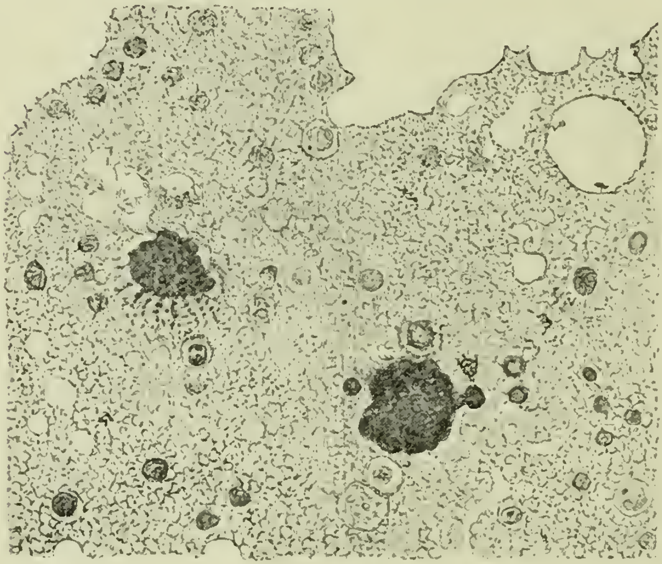


Fig. 32.—Didymium. Part of a stained section of a nodule similar in naked-eye characters to that shown in Fig. 9, Chap. I. The darkly stained bodies in process of growth by the flowing together of chromidial granules and nuclei are the pink oil-like drops of the unstained preparation. Drawing eye-piece. × 800 diams.

prepare us for unaccustomed features displayed in the illustrations. When we remember how long we overlooked the now obvious spirochaete phase of the protozoon of syphilis until a special mode of staining

smears and sections made it clear to all, we must not dismiss lightly apparently bizarre forms displayed by new methods; at the same time we may reflect that by suitable lighting, or by simply adding India ink, the spirochaete is better seen unstained. I wish the authors had given us an account of the appearance of the bodies they refer to in the living state.

The Mycetozoon they chose for comparison was *Badhamia utricularis*, of which the following account is given:—

> "The life cycle of *Badhamia utricularis*, though differing widely from the preceding, has certain close analogies to it. There are gametocytes and fully developed gametes. The method of conjugation does not appear to have been determined definitely, but it is probably direct, as in *Plasmodiophora*, without the preliminary formation of a microgamete. Conjugation is followed by a resting phase. When further development takes place, there is liberated from within a capsule a large rounded body with two long, thin flagella. It becomes a sporoblast. This discharges from its interior a large number of small rounded forms, which increase in size and give off rod-like bodies, or sporozoites, each of which has a flagellum at one end. These sporozoites may complete their development before rupture of the sporocyst. They next break up

into rounded nucleated bodies which represent an amœbula phase. It is very doubtful if there is any true plasmodium, though the term is commonly applied to the creeping film, which, however, is composed of independent sporozoites, amœbulæ, and gametocytes in various proportions, according to the age of the growth. The walls of the vessels that form an essential part of these films are apparently formed by deposition of successive layers of a pale type of sporozoite which is always present. The development of a sporangium marks the ripening of the gametes, the occurrence of conjugation, and the formation of resting spores. The important point in this life cycle for our present purpose is the occurrence of a rod or sporozoite phase. These rod forms appear always to have been mistaken for bacilli. We have made observations which we think place it beyond doubt that they are a phase in the life cycle of this protozoon. There is also evidence of the occurrence of an asexual cycle comparable to that described by Schaudinn in one of the malarial parasites."

Fig. 33 is the illustration of the provisional life cycle of Badhamia according to these authors.

I cannot help thinking that the authors have been misled in many particulars by trusting to

over-elaborate methods, and I hope they will someday revise their work. Some of the bodies they describe in human cancer and sarcoma, and in the infective sarcoma of dogs, seem to me to be the same as some of those described previously by myself. Their work deserves attention, and this would be greatly helped if the authors would supplement it by stating four things: how their bodies are related to the histogenetic part of cancer, how much of their life-cycle had been previously established in biology, how much of it can be traced in fresh living material and in sections prepared by the ordinary methods, and how their observations are related to those of previous workers in the same field.

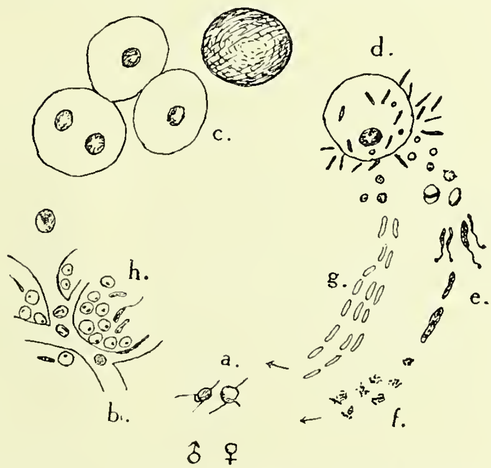


Fig. 33.—Ford Robertson and Wade's "Provisional Life-Cycle of a Mycetozoon.

Ford Robertson and Wade were the first to affirm that the protozoa of cancer are mycetozoa, and they also publish interesting experiments with human cancer material injected into mice.

It seems to me that the work of these authors shows that any one who looks seriously for protozoa in cancer will find them in abundance. I think the authors have succeeded in spite of, and not because of, their methods.

The protoplasm of Didymium, of the Mycetozoa of cancer, molluscum contagiosum, etc., has a peculiar density, and a quality which causes it to stain differently from the ordinary cells of human and other vertebrate tissues, and which, I doubt not, accounts for some of their forms having retained the silver precipitated in them by one of the methods employed.

Many details of the various stages require to be worked out before I could suggest any scheme as representing the complete life-cycle of Didymium.

The frequent occurrence of the stellate chromidial amœbula in mycetozoa is paralleled in cancer and sarcoma, as is shown in the following account of a choriocarcinoma, Chapter VIII., and as has been shown in Parts II. and III. of this work in an account of a sarcoma of the breast. Two illustrations of the latter are reproduced in Fig. 34.

The few points of comparison between phases of Didymium, on the one hand, and the protozoa of cystic ureteritis and cancer on the other, suffice to show that the biologist and the pathologist

might use the abundant material at hand in such morbid human tissues for the study of protozoan life with equal profit. In Chapter IX. further details

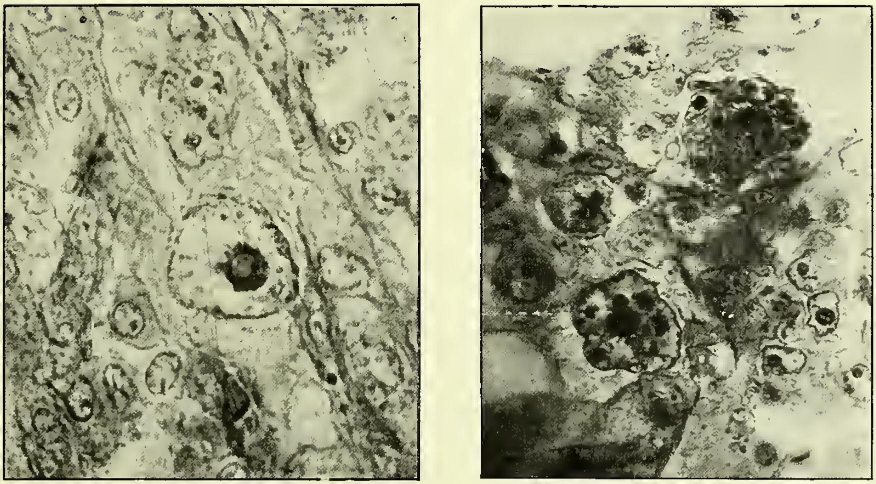


FIG. 34.—TWO MICROPHOTOGRAPHS OF AN ALVEOLAR SARCOMA OF THE BREAST. The one to the left shows a large intranuclear parasite, and that to the right at *b* shows a free parasite subdividing into stellate amœbulæ. From Part II. of "Protozoa and Disease." × 800 diams.

will be adduced to show that for the past sixty-eight years the mind of pathology has been hypnotised by false doctrine based on unwarranted assumption and imperfect knowledge.

5

CHAPTER V

MOLLUSCUM CONTAGIOSUM

Every practitioner of medicine is familiar with the lesions that are known by the name of molluscum contagiosum. They project like small pearl studs from the surface of the skin. In the middle is a slight dimple. In spite of the suggestion of softness in the name they are rather firm to touch, so that during epidemics of small-pox they are sometimes mistaken for the shotty stage of the eruption in this disease. They sometimes project more than usual, even hanging by a stalk, which may break, and so effect a spontaneous cure of individual lesions. More commonly when they have reached a certain size the dimpled centre gives way and allows a little cream-like fluid to escape. This is the moment when the local infection is apt to occur if any scratch or other breach of surface is present. The incubation period of experimentally inoculated disease is two months, and may be longer. The lesions have not always the pearl-stud character; where the skin is thick, as on

the hands, they may be mistaken for common warts, but a simple examination of a scraping under the microscope reveals the molluscum corpuscles; the superficial likeness of these to coccidia was long ago remarked upon. Fig. 35 shows them as they are

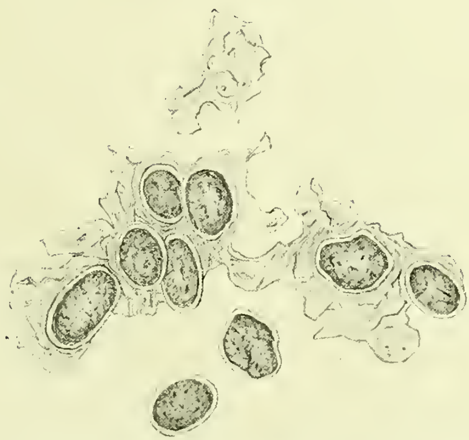


Fig. 35.—Molluscum Bodies and Squamous Epithelial Cells. From a teasing of a freshly excised nodule smeared on a slide and fixed with perchloride of mercury solution and mounted in glycerine and water. Drawing eye-piece. × 400 diams.

usually seen under a dry lens. When the lesions are too deeply placed to allow their contents to escape an inflammation occurs after a time, a small abscess resulting. Ulceration may follow, and such ulcers when occurring on the skin of the breast have been mistaken for cancer. If the disease is left to itself spontaneous recovery takes place in the course of a

year or two. If the little tumours are removed, and their bed is treated locally with a drop of nitric acid they heal, and do not recur at the same spot.

It is a question whether the disease arises in every case by local inoculation. In the corresponding disease in birds it has been found that an emulsion of the molluscum bodies given by the mouth causes the disease to appear in the skin at points where the latter has been injured, *e.g.* by pulling out feathers. I have seen a child as thickly covered with molluscum tumours as is a person with a bad attack of discrete small-pox.

The virus has been found to be filterable through a porcelain filter, and it has been made by V. Prowazek, one of the chief elements in the foundation of his theory of "Chlamydozoa" (see below, Chapter X.), which the observations here published show to be untrue as far as molluscum is concerned.

The therapeutic application of X-rays has been found to cure this disease. This is interesting in itself, and also in connection with the fact that a few cases of cancer have been cured by the same means. As an instance, with the kind permission of the author, the case reported by Lidbrooke F. Cope[1]

[1] *The Lancet*, June 5, 1915.

may be given. It also shows the effect of this infection on the pigment of a negro's skin.

"The patient, a Krooman, was admitted to the Royal Naval Hospital, Simon's Town, with a group of little tumours situated just above the right elbow-joint (Fig 36, *a*). Each tumour contained a soft central core from which there was an exudation consisting of serum, a few erythrocytes and cocci, together with the typical hard oval 'molluscum bodies.' In the occipital region he had a small hard tumour of the keloid type. The history was indefinite, the only information the patient could give being that he had noticed the place on his arm 'for some time.'

"Treatment in the first instance consisted of fomentations with 20-grain doses of potassium iodide thrice daily, without any improvement whatever. After ten days of this treatment it was decided to try the effect of X-rays. The affected part was accordingly exposed to X-rays for ten minutes every other day. The condition at once began to improve, and at the end of the twelfth sitting had completely healed. The second photograph (Fig. 36, *b*) was taken fifteen months later, and gives satisfactory evidence that the cure is permanent.

"The chief points of interest in the case lie in the fact that in the pigmented skin of the

a

Fig. 36, a.—Condition of the Arm before Treatment with X-Rays.

b

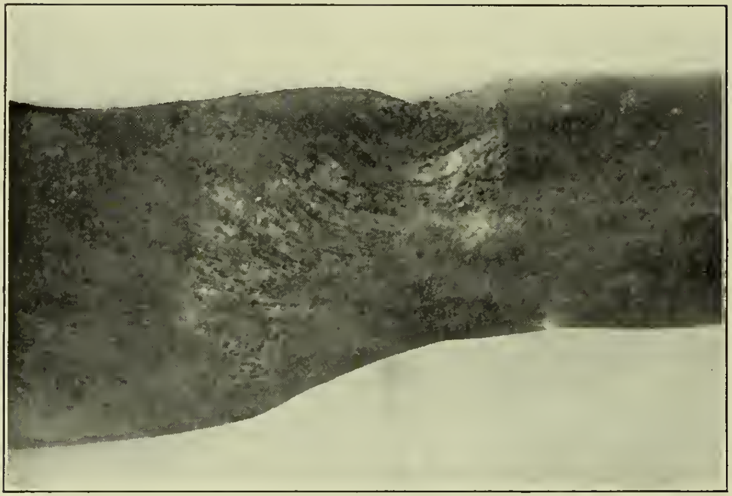


Fig. 36, b.—Condition Fifteen Months after Treatment.

Reproductions of Photographs illustrating Staff-Surgeon L. F. Cope's Note on a Case of Molluscum Contagiosum cured by X-Rays.

negro it is not easy to see zones of inflammation, etc., such as give characteristic appearances to skin lesions in Europeans, and the rapidity with which the condition healed with X-rays—a result quite in accord with those obtained by Norman Walker and other dermatologists."

The pathological view.—Molluscum contagiosum is placed in systematic works on the pathology of the skin among anomalies of cornification or dyskeratoses.[1]

[1] The chief features of "Keratinisation" are given as shown in the following extracts from a work by J. M. H. McLeod, on the "Pathology of the Skin," 1903 :—

"Towards the surface, the prickle-cell layer of the epiermis merges into a layer consisting of two or three rows of flattened cells, containing numerous granules. . . . The granules of the cells are densest in the protoplasm near the nuclear space. . . . To avoid the multiplication of terms, the name 'Keratohyalin' is generally retained for these granules, while the name 'eleidin' is applied to an oily fluid substance which occurs in and between the cells of the *stratum lucidum.* Keratohyalin is distinguished from Keratin by being digestible in pepsin and soluble in strong acids.

"The most perfect horn-cell is situated immediately above the *stratum lucidum.* It presents a space in the centre from which the nucleus has completely disappeared. The eleidin and hyaloplasm of the cell have given place to a fatty or waxy substance, and the peripheral portion of the spongioplasm has become transformed into a highly resistant substance named keratin. The epithelial fibres or prickles persist as dried shrunken spicules, having also become changed into keratin. The existence within the horn-cells of fat makes the stratum corneum a waterproof coating to the body.

> "The majority of writers now consider them to be horn-cells, which are peculiar, in that they contain a homogeneous mass of colloid or hyalin (a degenerative product of the protoplasm of the cell) instead of the usual waxy contents. The molluscum bodies are described as consisting of a capsule within which there is a more or less oval homogeneous mass, and squeezed between it and the periphery there is a flattened nucleus, or remains of the nucleus."

In this description the origin of the cell-inclusion within the nucleus is overlooked, and there is no mention of the definite real capsules so frequently formed from the contained parasite as distinguished from the remains of the epithelial cell.

Keratin can withstand 50 per cent. mineral acids for a long period. It is indigestible in pepsin-hydrochloric acid. It is soluble in weak alkalies.

"'Dyskeratoses,' or anomalies of cornification, are made by dermatologists to include the *Psorospermosis follicularis vegetans* of Darier and molluscum contagiosum.

[The former is practically a discrete cancer of the hair follicles, and as shown in Chapter V., molluscum bodies are protozoa; and I have little doubt that the bodies described as psorosperms will prove to be the same as the protozoa of other forms of cancer.—J.J.C.]

Concerning the molluscum body, McLeod writes: "The majority of writers now consider them to be horn-cells, which are peculiar in that they contain a homogeneous mass of colloid or hyalin (a degenerative product of the protoplasm of the cell) instead of the usual waxy contents."

PLATE III

A Vertical Section through an entire Molluscum Tumour. The whole has an Ʊ-like form. The horizontal limbs of the Ʊ are normal skin with its papillæ flattened by pressure. In the middle is an enlarged papilla; the cells of the superficial layers are directed vertically by reason of the abnormal pressures to which they have been subjected. The interpapillary epithelium is increased in amount in the tumour itself, and some of the stages in the formation of the molluscum bodies can be seen in them. Drawing eye-piece. × 100 diams.

[*To face p. 73.*

The confusion of epithelial host-cells with the parasites may in a measure be understood by looking into the details drawn in Plate III. The central papilla has a lucid layer as well as a horny layer, the cells of the latter by abnormal pressures being forced into a vertical instead of the usual horizontal position. Following with the eye the lucid layer laterally it is noticed that the molluscum bodies that occupy the place of the lucid layer are clearer, less stained, and more highly refracting than those on either side of them. Further, the molluscum bodies that occupy the place of the horny layer like the cells of this layer, are somewhat indefinite in appearance. But it is only to be expected that parasites that have fed on the protoplasm of a cell should to some extent share in the physical and chemical properties of the cell that it inhabits. Many details of structure described above, such as the chromatic chromidial strands, are overlooked entirely by systematic writers.

Histology.—A tumour taken at the moment of discharging its contents fixed in a good fixing medium and stained with dyes of well-known reactions [1] under a magnifying power of 100 diameters,

[1] This tumour, like the mycetozoon described in Chapter IV., was fixed in a solution of equal parts of saturated perchloride of mercury made in boiling saline solution, and 5 per cent. solution of bichromate of potassium; the solutions are mixed at the time of using. The stain, acid hæmatoxylin and eosin.

has the features shown in Plate III. The tumour is an overgrowth of all the elements of the skin over an area the diameter of which is seen as the gap between the two portions of normal skin at the uppermost part of the drawing. It is the epithelial part of the skin that is mainly overgrown. In pathological language, then, the little tumour is an epithelioma. The fibrous tissue that supports the overgrown epithelium and contains the blood-vessels, which convey nourishment and remove waste products, is clearly seen, and it is nowhere penetrated by the epithelial cells. The tumour is therefore of what is known as the benign type.

A simple microscopic examination such as this is the method employed for the diagnosis of tumours in the daily routine of medical practice. It is of great service in this respect. The same superficial examination is also of use in forecasting the course a tumour is likely to run. Long practice of this simple method enables us to recognise at a glance certain points; *e.g.* the tissue from which the tumour grows, whether the new growth is shut off from adjoining tissues by a capsule, or whether its cells infiltrate, that is extend along the lymph-vessels or other channels—in other words, whether it is benign as is the molluscum tumour we are considering or malignant like a cancer or sarcoma. There are types

of cell, *e.g.* the small round cell in sarcoma to which experience has attached a character of high malignancy, whilst, among sarcomas, the "giant-cell" or myeloid type is often almost benign.

Examined more minutely the stages of formation of the cell-inclusions or molluscum bodies can readily be followed as shown in Fig. 37, *a*. The first recognisable stage is a minute non-nucleated stellate body,[1] which stains like the elements of the mycetozoan mycelium, *i.e.* like a protozoon in the chromidial state. This and the succeeding stages are clearly shown in Fig. 37, *b*, *c*, *d*, *e*.

A general comparison of the stages here met with and corresponding stages of Didymium may be made, differences owing to the parasitic habit of the molluscum body being necessary, and also for incompleteness in our knowledge of both organisms.

Under an oil immersion lens the molluscum bodies and the remains of the nucleus of the containing cells show more distinctly the characters noted above as seen with a dry lens. This is appreciated by comparing Fig. 37, *a*, with Fig. 38.

Evidences of vitality.—The appearances which I have observed in molluscum bodies kept

[1] This observation I made first in 1894, and it was published with an illustration in the *Centralblatt für Bakteriologie*, February 28, 1895.

Fig. 37.—*a*, Part of a Section of a Molluscum Tumour. Various stages of the molluscum bodies are shown in the molluscum bodies, in most of them chromatin in the form of chromidial granules is present; *b*, The earliest stage, a non-nucleated stellate amœba within the nucleus of the epithelial cell; *c*, Two amœbæ between the nucleus and the cytoplasm of an epithelial cell; *d*, The molluscum body is larger and consists of a plasmodium of extremely small stellate amœbæ; *e*, A still larger parasite; the individual amœbæ have fused together and the body is finely granular. *a*, × 600 diams., the rest × 800 diams. Drawing eye-piece.

in water or nutrient liquids are: 1, segmentation; 2, protoplasmic commotion; 3, the formation of a supporting framework comparable to that described

Fig. 38.—Part of a Section more highly Magnified. The chromidia are well seen, also the subdivision of the parasites into rounded segments. One contains a vacuole. Drawing eye-piece. × 800 diams.

above in a mycetozoon; 4, the formation of separate globular bodies having streaming protoplasm; 5, formation of minute bodies containing vacuoles which change in size; 6, formation of flagellate bodies; and

7, formation of oil-like subdivisions. These several features will now be considered in detail.

1. *Segmentation.*—This concerns the whole of the corpuscle except the cortical layer. The segments are angular in form. In preparations made in tap water nearly every corpuscle in the field of a one-eighth inch objective may be found to be affected by it. The segments are granular, and that they result from a vital process is shown by their sometimes becoming rounder, and their protoplasm becoming involved in the process next to be described. This change may affect all the subdivisions or some of them, or even only one: see Plate IV., Fig. 3.

2. *Protoplasmic motion.*—This important feature I have obtained very often from the third to the fifth days in cultures. It is seen both in corpuscles with and without supporting framework, and in others in which this has formed incompletely, and also in smaller spherical bodies. The corpuscles or parts of corpuscles in which streaming is taking place become clearer, and their granules very small, so that a good north light[1] and very careful focussing are required to follow it. I have been able to trace granules through several compartments of a corpuscle in which

[1] The light from an electric lamp is very apt to deceive as to movement: the lighting is particularly important when vital processes are being looked for.

a supporting framework had developed. The framework itself, even when it is in the form of isolated segments, is unaffected by it, and any coarser granules that may be present sway or roll something like a light boat anchored in a stream. These features are indicated in Plate IV., Fig. 1, *a*, *b*, *c*, and Figs. 2, 3, and 6.

3. *Supporting framework.* — This, when complete, is a definite highly refracting coarse net, continuous with the cortex of the corpuscle (Plate IV., Fig. 1, *a*). In other instances it is represented by a series of segments apparently unconnected with one another (Plate IV., Fig. 2). An occasional feature, and one not without interest in view of the frequent formation of highly coloured substances by various mycetozoa, in some cultures what I take to be the first-formed portions of the framework have a bright rose colour. The special capsules (Plate IV., Figs. 3, 7, 9, 10) may be regarded as part of the supporting framework.

4. *Spherical bodies*, smaller than the typical molluscum corpuscle with streaming protoplasm, are very common; sometimes they have an ectosarc (Plate IV., Figs. 3 and 6). When the streaming ceases they are indistinguishable from the oil-like drops described under heading "7."

5. *Vacuoles.*—Two examples are shown in

PLATE IV

Molluscum Contagiosum. Bodies seen in a teasing of a bacteria-free culture on the fourth day. Fig. 1: *a*, A body with framework developed and which showed protoplasmic movement throughout; *b*, a body similar to *a*; *c* showed protoplasmic movement in part only of its extent; its framework is imperfectly formed; *d*, a body which showed a framework but no motion; *e*, one of three minute motile bodies; *f*, highly refracting globules; *g*, epithelial cell. Fig. 2, Body with imperfectly formed framework which had all its protoplasm in motion, and a minute body escaping at a gap in the contour. Fig. 3, Molluscum body segmented, the protoplasm of one segment only in motion; smaller spherical bodies, two with vacuoles and two with protoplasmic motion. Fig. 4, Two zoospores with oscillating granules and molluscum body with similar moving granules. Fig. 5, Molluscum body extruding globules similar to those in 1, *f*. All the foregoing drawn by the aid of the drawing eye-piece; × 800 diams. Fig. 6, Four molluscum bodies and an epithelial cell as seen with a dry lens; one molluscum body showed protoplasmic motion. Fig. 7, Four molluscum bodies, one encapsuled, another segmented. Fig. 8, Molluscum body encapsuled within an epithelial cell. Fig. 9, Similar to 8, but showing a globule being extruded. Fig. 10, Encapsuled molluscum body, bacteria outside but not inside the capsule. Fig. 11, Unencapsuled molluscum body segmented and invaded by bacteria:—this is the end of a culture that is unsuccessful. Figs. 6 to 11 × 400 diams.

PLATE IV

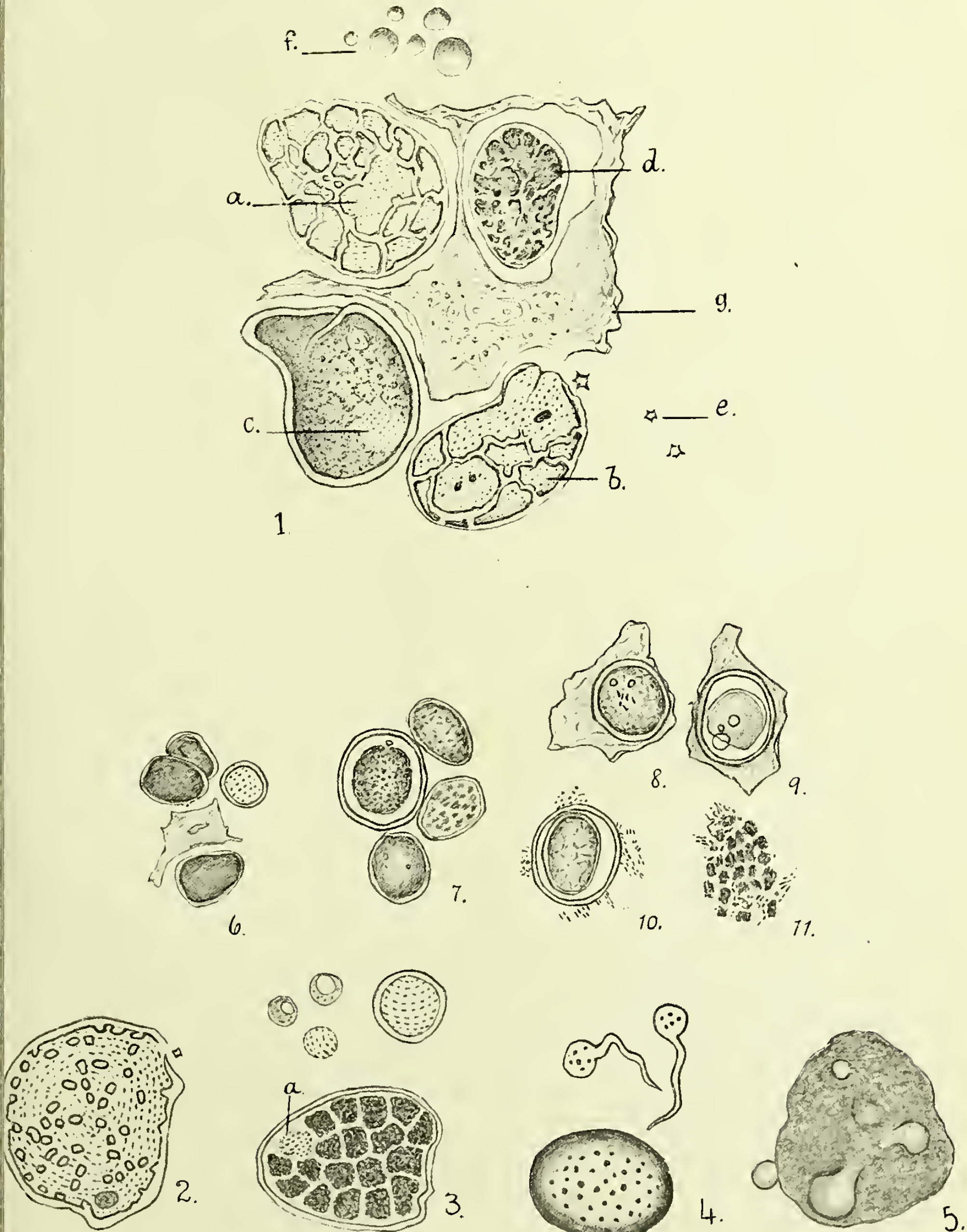


Plate IV., Fig. 3. They can sometimes be seen to become larger or smaller. I have not as yet seen one disappear suddenly like the contractile vacuole of many rhizopods.

6. *Flagellate bodies resembling mycetozoan zoospores.*—This striking phenomenon I have only once observed in a culture on the fourth day. The material was from abundant lesions, some of which I heaped up in the hollow of one of the cupped slides for hanging-drop observations, and placed in a moist chamber. The result I described in the *Centralblatt für Bakteriologie*, 1895, vol. i., p. 245.

The description (translated) runs—

> "The most remarkable appearance consists in the presence of a great number of actively-moving flagellate bodies. They have a roundish head of the size of a red blood corpuscle, and a single powerful flagellum, and under a one-twelfth immersion lens were easily seen and unmistakable; many passed across the microscopic field and then escaped from sight. Many of the molluscum bodies were unchanged; of others, but a thin bacterium-filled shell remained; still others had apparently undergone a liquefaction in their central part, and in this area numerous highly-refracting fragments in oscillating movement (Plate IV., Fig. 4), such as one not only

sees when unorganised particles are suspended in liquids, but also in many living protozoa.

"I may add that in what I called the heads of moving bodies (see Plate IV., Fig. 4), there were oscillating particles quite like other oscillating particles present in some of the molluscum bodies. The room in which the culture was made, in March, would be at a temperature not much above freezing at night, and by day somewhat above blood-heat, the preparation being placed on a chimney-piece over a bright fire."

Oil-like subdivisions.—A last evidence of vitality is the formation of great numbers of greenish globules. They resemble similar bodies in vaccine lymph. Although they look like globules, such as we see in cream, they do not behave like oil drops, which in water-mounted preparation rise to the surface and lie next to the cover glass, at the same time they have the appearance of high refraction often seen in bodies rich in lipoids. The mode of their origin is shown in Plate IV., Fig. 5. I regard them as a phase of the protozoon in a complete chromidial state. When a globule in protoplasmic movement comes to rest it assumes the same appearance as these fat-like globules.

I will add a few details to the foregoing account of vital phenomena in molluscum bodies, so

that any one accustomed to observe living protozoa may be able to repeat them.

Protoplasmic motion.—On many separate occasions I have notes before me of observations made in different years, from 1894 to the present year, and in different months, from February to November. I will describe first the conditions in which I obtained the movements observed in a preparation or culture free from bacterial contamination, Plate IV., Figs. 1, 2, and 5. The material consisted of one typical lesion I excised from the forehead of a boy, after cleansing the skin thoroughly with ether and spirit soap, and afterwards with sterilised water. A culture tube of a weak infusion of tea, made by boiling a piled teaspoonful of a large-leaved kind of tea taken from an infuser after having been once used and boiling it in six ounces of tap water for ten minutes on three successive days. To this infusion two per cent. of sterilised milk[1] was added.

[1] It is probable that the medium would have succeeded just as well without the milk, the cream of which rising to the water-level might be mistaken for a growth of bacteria. The temperatures are recorded, but I have found that there is a considerable margin in which the vital phenomena occur. The tightly-plugged culture-tube and the even temperature of the autoclave are not suitable for culture of these protozoa. The idea of adding milk to the medium was suggested to me by reading an article by C. O. Miller on "The Aseptic Cultivation of Mycetozoa" in the

On the 19th May, 1915, the growth was put immediately after excision in a sterile tube and transferred to the culture-tube an hour later by a sterilised wire loop. In the course of being transferred it became divided into two parts, which were placed on the surface of two separate tea-leaves in the same culture-tube (see Fig. 39). The tube was closed by four layers of sterilised gauze in order to allow access of more air to the culture than does the ordinary plug of cotton-wool. The preparation was kept in room where the temperature, about 9 a.m., was on the 19th and successive days 53° F., 50° F., 61° F., 67° F.

On the 23rd the preparation was kept for two hours at 90° F., and then placed in bright sunshine for ten minutes before, at noon, they were teased out in a drop of sterilised tap-water on a slide cleansed with absolute alcohol. The exact conditions are given, though from previous experience I know an approximation to them would succeed. Very many of the large typical molluscum corpuscles showed the lively commotion of protoplasm which I first described in the Appendix to Part II. of this work, 1908.

It follows from the observations recorded

Quarterly Journal of Microscopic Science, New Series, vol. 41, 1898, p. 43.

above that if molluscum bodies are subjected to cultural conditions similar to those that succeed with a common mycetozoon, some of them pass through changes which amply prove them to be organisms closely related if not actually belonging to the mycetozoa. The epithelial cells do not pass through any of the changes described. This proof is of far-reaching importance, showing the Chlamydozoa theory of V. Prowazek to be unsound, and the assumption that a filterable virus is necessarily ultra-microscopic to be untenable. The proof also carries with it the protozoan nature of the kindred bodies in cancer and sarcoma, and the protozoan nature of these diseases.

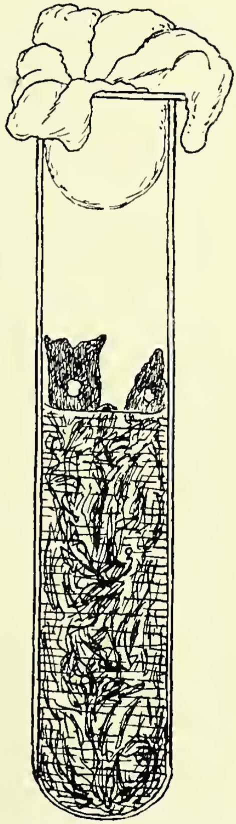


Fig. 39.—A Test-tube containing a Weak Infusion of Tea with 2 per cent. of Milk added. Two leaves project above the surface of the liquid, and on each of them, above the surface of the liquid, is placed part of the contents of a molluscum tumour.

Definite vital phenomena such as those just described in molluscum bodies are incontrovertible. The extraordinary power of resistance to the action of

strong chemical reagents and digestive fluids that Török and Tommasoli[1] found these bodies to possess does not prove them not to be protozoa, but it now gains a new significance. I have little doubt that in some of their phases the mycetozoa will be found to be equally resistant, and as soon as I can find an opportunity I hope to test this point.

If the vital processes were not as definite and clear as they are, control observations might reasonably be asked for. As a matter of fact, the epithelial cells present in successful cultures themselves afford a control: they are seen to be unaffected by the streaming movements and other vital changes described above. The evolution of the horn-cell—its loss of nucleus, etc.—approach, in superficial appearance at least, the change from the nucleated to the chromidial state in a rhizopod; and a recticular structure has been described in cells in certain horny tumours. In the latter case mycetozoa that have developed an internal framework may have been mistaken for horn cells.

Molluscum contagiosum of birds.—This affection has already been mentioned, and some further mention of it will be made in a later chapter. It is probably one aspect of an infection of which the flagellate diphtheria of birds is another. My own

[1] "Monats. f. prakt. Dermatologie," Part X., p. 149, 1890.

observation of flagellates in a culture of human molluscum lends this idea some support. A very extensive literature exists on the subject. I have not seen any record of cultivation experiments made on lines suitable for mycetozoa, and I think these would probably prove the protozoan nature of avian molluscum as my own have proved the same for the human disease.

CHAPTER VI

CHROMIDIA

To understand the nature of chromidia some reflections upon the fundamental characters of the protozoan nucleus are necessary. Non-nucleated protozoa exist, but in them *chromatin* is present in the form of chromidial dust or granules. The nucleus has a dual function, as is most plainly seen in infusoria where the macronucleus is the trophonucleus and the micronucleus is the gonad or sexual nucleus. In many protozoa the two parts are contained in one nucleus, separating only in preparation for fertilisation.

Some nuclear processes in Amœbina.—The common rhizopod, *Amœba proteus*, the asexual binary division of which is demonstrated in every laboratory, exhibits vital processes about which biological opinion is divided. There is, it appears, a sexual generation which is thus described by Calkins.[1] The sexual

[1] Gary N. Calkins, "Fertilisation of Amœba Proteus," *Biological Bulletin*, Sept. 4, 1907.

cycle begins by multiplication of nuclei till many *primary nuclei* are formed. All these except one, which remains as a residual nucleus,[1] fragment into minute granular *secondary nuclei.* These secondary or gametic nuclei fuse together in pairs, forming fertilisation nuclei, in which the joint nucleoli break up into fine powder, which gathers in foci at the periphery forming *tertiary nuclei*, each of which probably constitutes the nucleus of a pseudopodiospore (see Fig. 40, *g*, *h*). It may be noted that if the breaking-up of the nucleoli into chromatin powder were followed by this powder leaving the nucleus to form nuclei in the cytoplasm, it would constitute a chromidium as defined below. In the alternative view the processes just described are referred to parasitic infection similar to that described on p. 98 under the head of Cryptodifflugia.

Microgamete formation in Coccidia.—The early microgamete stage of *Coccidium oviforme* which I described as long ago as 1893,[2] affords another illustration of a nuclear process which approaches chromidium formation in character; the process is

[1] Calkins compares the primary, residual, and the secondary nuclei of Polystomella with these in *A. proteus.* The secondary nuclei are equivalent to the sporozoan sporoblasts, as they were named by Schaudinn.

[2] *Medical Press and Circular*, Sept. 20, 1893, p. 297.

illustrated in Fig. 41. The minute motosis in the first of these may be a reduction division.

Chromidia.—The term "chromidium," which now denotes the whole collection of extranuclear

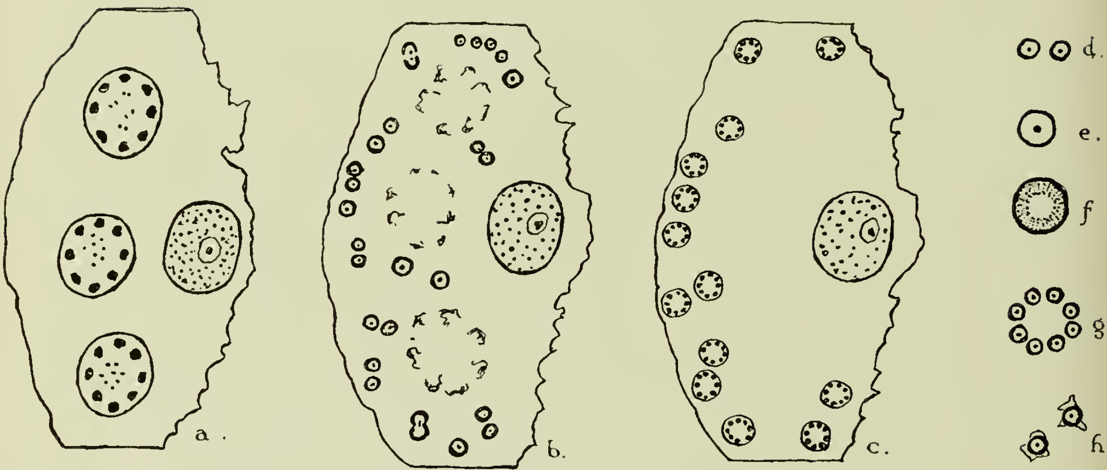


FIG. 40.—*a*, *b*, *c*, THE SAME PORTION OF AN AMŒBA PROTEUS SHOWING STAGES IN THE SEXUAL CYCLE OF CALKINS. *a*, Three primary nuclei and the residual nucleus; *b*, Secondary nuclei formed by chromatin fragments leaving the primary nuclei; *c*, Tertiary nuclei formed by fusion of primary nuclei or gametes in pairs. Details of these processes: *d*, Two primary nuclei; *e*, Zygote formed by fusion of the foregoing; *f*, Nucleolus breaking up into fine particles which collect at the periphery; *g*, Spores in tertiary nucleus or sporoblast; *h*, (probable) Amœbulæ or pseudopodiospores. Diagrammatised after Calkins.

chromatin present in any one organism, was first applied to the individual grains of extranuclear chromatin. Minchin suggests that the individual elements of the chromidium be called "Chromidiosomes," but

where the condition is very fully developed no separate grains of chromatin can be recognised under the microscope, the nuclear and cytoplasmic elements being so intimately mixed together. Goldschmidt, as stated by Doflein,[1] has described Chromidia in the tissue cells of many metazoa. To the contrary, on p. 66 of his work Minchin[2] has written: "In the tissue-cells of Metazoa, as a general rule, and in many

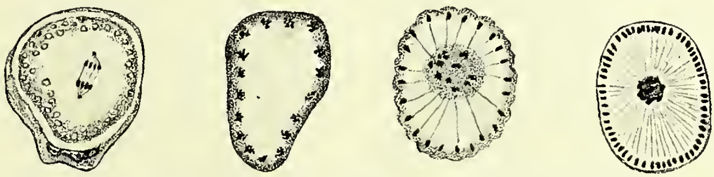


FIG. 41.—STAGES IN THE FORMATION OF MICROGAMETES IN COCCIDIUM OVIFORME. From *Cent. f. Bakt.*, Feb. 28, 1895.

protozoa the chromatin is concentrated entirely in the nucleus or nuclei and chromidia do not occur."

It may prove that the more specific products of the highly specialised cells of metazoa, for example, the secretion granules or drops of gland-cells, are chromidial in their origin. The mammalian red blood corpuscle in the manner in which its nucleus disappears suggests a chromidial process.

Chromidia were originally described by R.

[1] Doflein, J., "Lehrb. d. Protozoenkunde," 1909.

[2] Minchin, E. A., "An Introduction to the Study of the Protozoa," 1912.

Hertwig,[1] as occurring both in normal and pathological conditions in the radiolarian Actinospherium. When nutrient matter is withheld in cultures the nucleus becomes dissolved, and is changed into chromidia,

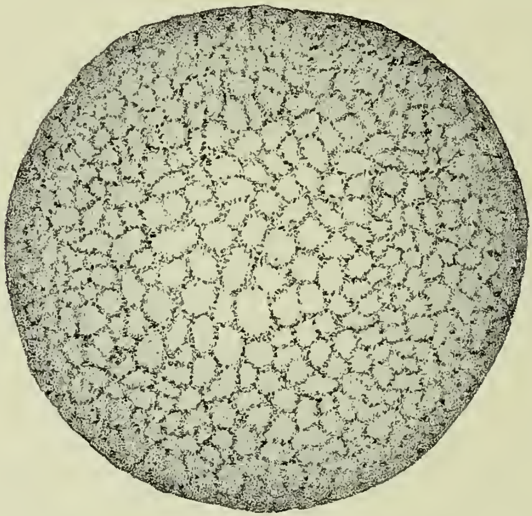


Fig. 42.—Actinosphærium in the Chromidial Condition. After R. Hertwig.

which pervade the plasm of the animalcule, which becomes darkly stained throughout (Fig. 42).

My own studies of Chromidia[2] began long before the condition had received a name in biology.

[1] R. Hertwig, "Die Protozoen und die Zelltheorie," *Archiv. für Protistenkunde*, vol. i., 1902. R. Goldschmidt, "Der Chromidien der Protozoen," *Arch. f. Protozoinkunde*," vol. v., 1904, p. 126.

[2] "Morbid Growths and Sporozoa," 1893, p. 42.

In 1892, in the protozoa of cystic ureteritis, I examined animalcules that appeared to be able to create nuclei from their plasm, which stained equally with nuclear and cytoplasmic stains. In various cancers and sarcomas similarly organised bodies claimed my attention.

Of one of the cells which I regard as protozoa in a cancer, I wrote: "This phase of the parasite seems thus to be due to a generalisation of the chromatin preparatory to subdivision."

Some examples of Rhizopods in which choromidium-formation occurs may now be considered.

Mastigella Vitrea.—Protozoa which combine the characters of the Flagellates with those of the Rhizopods are known as the Rhizomastigina. The study of their life-history led biologists to place them rather among the rhizopods than in a group connecting the cercomonads with the rhizopods. Several authors have pointed out their close relations with the mycetozoa. The diagram (Fig. 43), indicates the life-cycle of one of this group. There is a flagellum, and in some genera two or more flagella. The body of the animalcule is capable of extensive amœboid changes of form. The outer layer (ectosarc) in some species is furnished with bristle-like or other appendages. The flagellum arises in the plasma

where its origin may be closely associated with the nucleus. Nutrition is holozoic, *i.e.* by inception of solid particles. A contractile vacuole is present. In

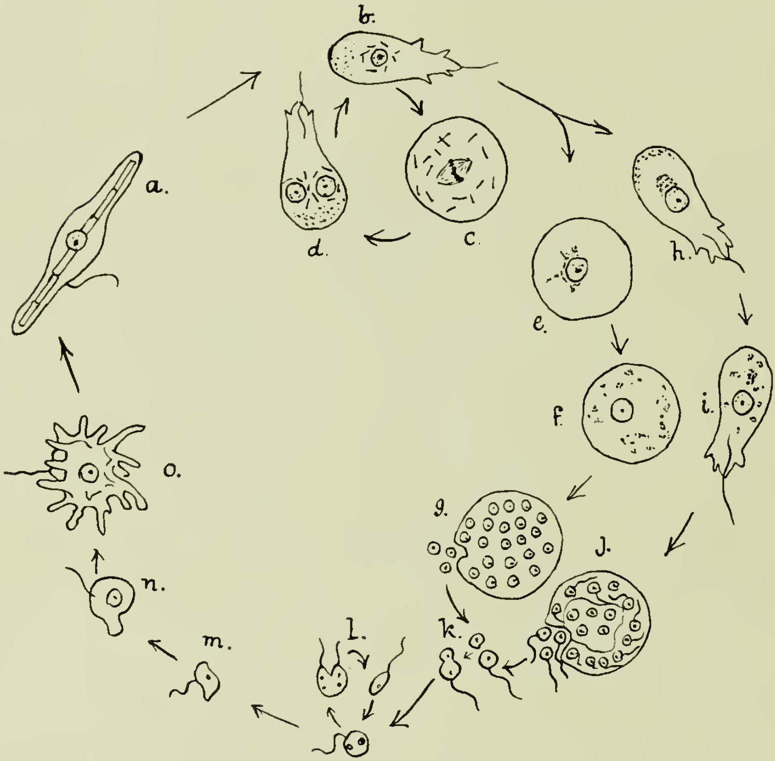


FIG. 43.—THE LIFE-CYCLE OF MASTIGELLA VITREA. Modified after Goldschmidt.

the amœba-like stage the nucleus is of the ordinary type with nucleolus.

In the freely moving form the animalcule becomes elongated, and a number of rod-like bodies

appear around the nucleus (Fig. 43, *b*). Asexual multiplication takes place in this state, the animalcule becoming rounded, and the nucleus dividing by karyokinesis (Fig. 43, *c*, *d*). The sexual generation is produced by free nucleus-formation from the chromidium within the plasm of the mother-cell (Fig. 43, *e*, *f*, *g*; *h*, *i*, *j*). The original nucleus remains unchanged for a time and then disappears. The gametocytes become encysted at the same time that the chromidium is formed (Fig. 43, *g*, *j*). Macrogametes and microgametes are produced from different mother-cells, the former alone are flagellated. The zygotes are flagellated, and they also are capable of longitudinal subdivision: metagamous reproduction. The adult form is assumed by growth and the protrusion of pseudopodia. A chromidium of *Mastigella vitrea* on a larger scale is shown in Fig. 44, together with kindred stages in a coccidium and a cancer protozoon. The formation of the chromidium in mastigella occurs without the disappearance of the nucleus, from which part of the chromatin escapes and forms a dense chromidial mass in the plasm near the nucleus. The chromidium grows and new nuclei gradually become differentiated from it, and escaping from the mass spread as gametes among the meshes of the plasm till the latter becomes filled with them. This phenomenon of free-cell

formation is of the highest importance in protozoan life, in rhizopods especially

Cryptodifflugia.—Another instance may be given, one that illustrates both chromidium forma-

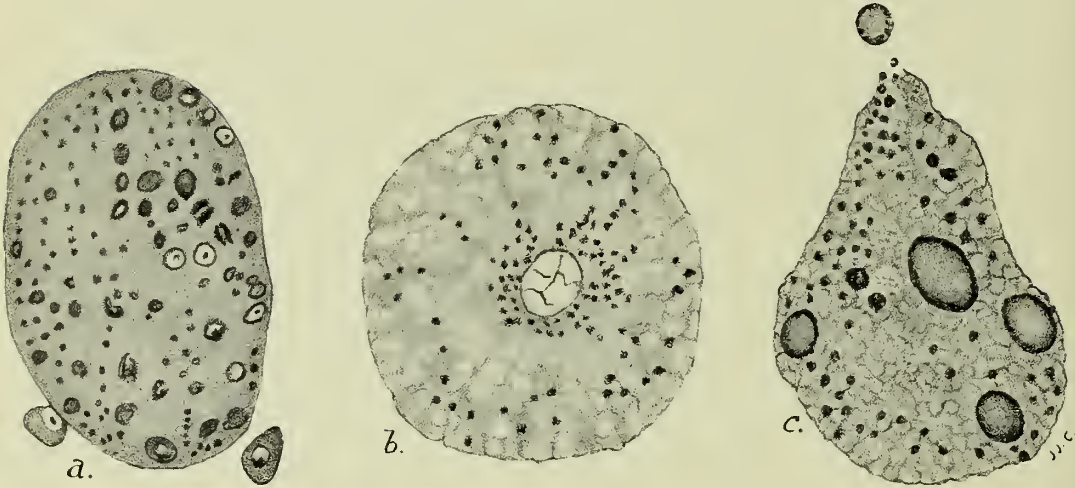


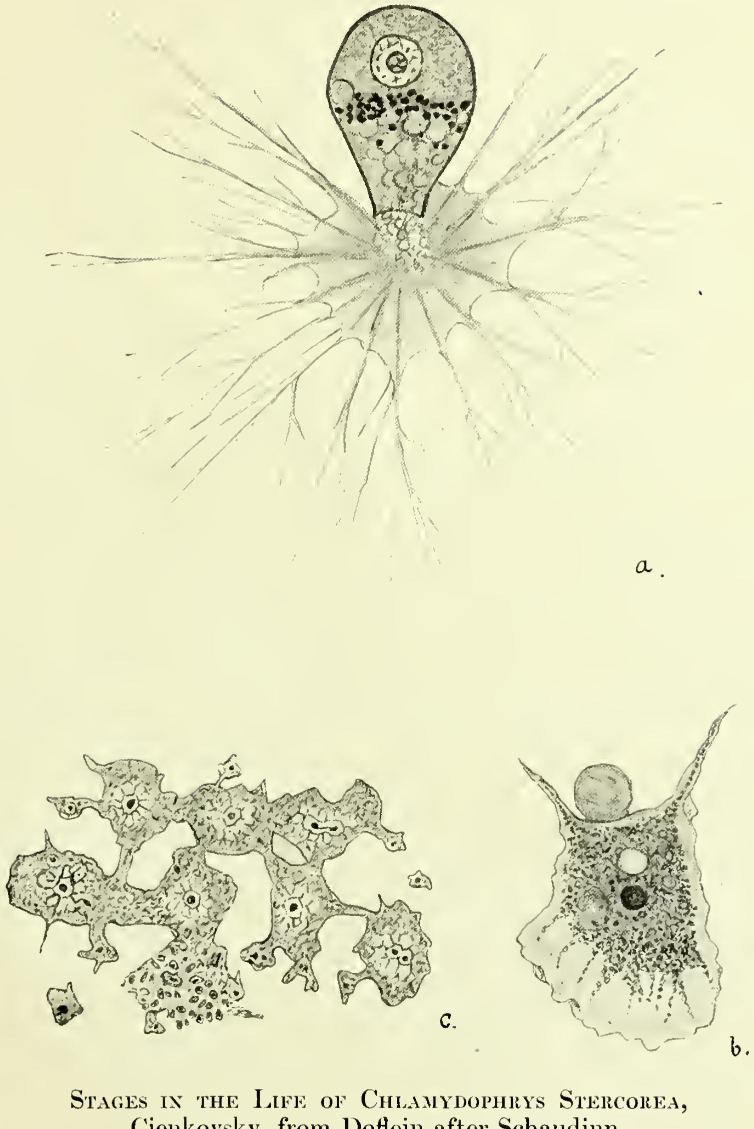
FIG. 44.—*a*, Gametogenetic chromidium of *Mastigella*; *b*. Chromidial microgamete nuclei in a *Coccidium*; *c*, Chromidium from the Choriocarcinoma described below in Chapter VIII.

tion and parasitism of one rhizopod protozoon upon another. The small monothalamian foraminifer, Cryptodifflugia (Plate V.), according to Prandtl's observations, when incepted by *Amœba proteus*, and when it happens to be ripe for its sexual process, is able to resist the digestive juice of its host in the body of which it undergoes a series of changes. The

PLATE V

Cryptodifflugia. *a*, A shelled state; *b*, Asexual multiplication in the shelled state; *c*, Sexually ripe state, the animalcule leaving its shell, the nucleus is now of the chromidial type; *d–i*, The series of changes passed within *A. proteus* ending in isogametes being formed from the Cryptodifflugia which is in the chromidial condition; *j*, *k*, *l*, Conjugation; the zygote; *m*, is biflagellate; *n*, Multiplication in the flagellate state; *p*, Flagella disappear, the animalcule becoming amœboid previous to the development of its shell. Modified from Doflein.

PLATE VI



Stages in the Life of Chlamydophrys Stercorea, Cienkovsky, from Doflein after Schaudinn.

brood of gametes escapes into the water to unite in pairs, and after passing through a bi-flagellate phase in which meta-sexual reproduction may occur, the animalcule changes to the free-living foraminifer state. It would appear that the sexual stages can occur either free in the water or in the body of *A. proteus.*

A study of this monothalamian foraminifer affords but one more of the many examples of how complicated the life-history may be among protozoa.

Chlamydophrys stercorea.—Another example of a rhizopod possessing a well-marked chromidium is seen in *Chlamydophrys stercorea* described by Schaudinn. It belongs to the monothalamian fresh-water foraminifera, and has a degraded parasitic as well as a free-living form (Plate VI.). Its parasitic phase, or Leydenia form, has been made prominent by its having been met with in ascites fluid in a case of human abdominal cancer; in this phase it was termed *Leydenia gemmipara.*

Chromidia and alternation of generations in Foraminifera.—One of the most important biological discoveries of recent years was made independently by J. J. Lister and the late Fritz Schaudinn. It concerns the Foraminifera, and though established as yet only for certain species, may probably prove to apply to the whole order. The fact that has been established is the existence of an alternation of

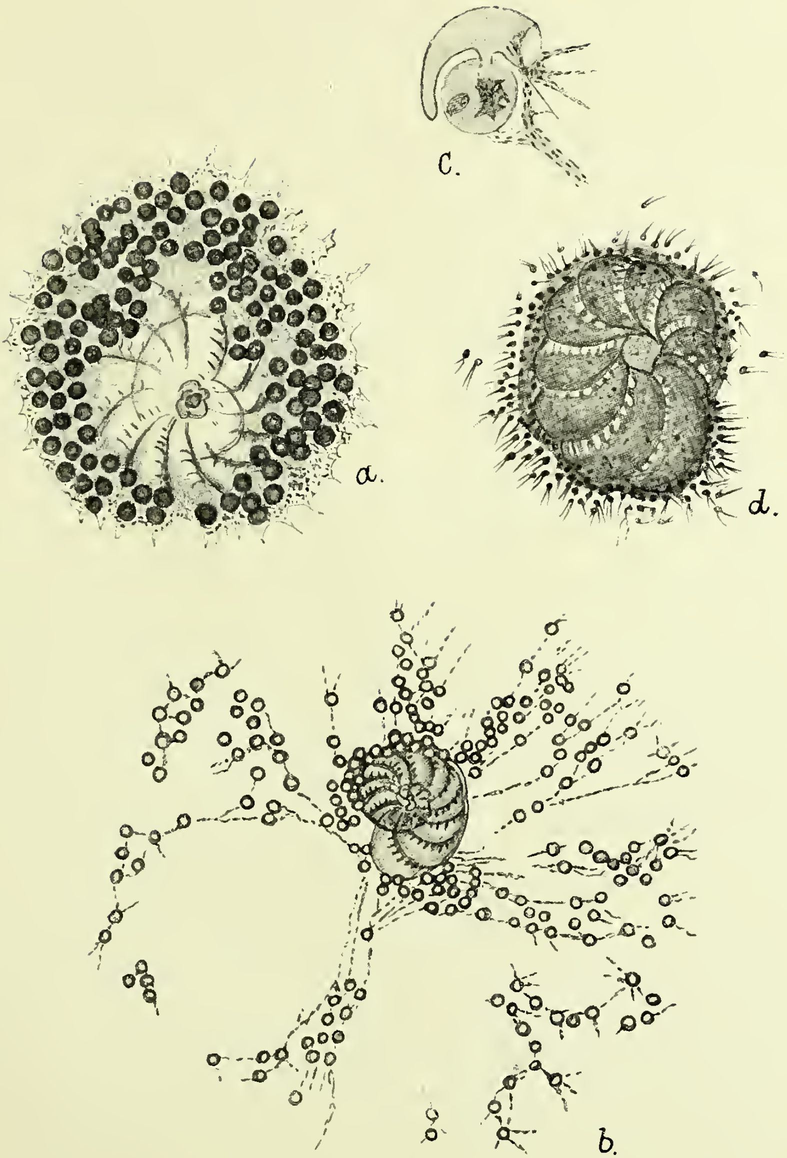
a sexual with an asexual generation. Lister established this for one of the imperforates, Orbitolites, and for one of the perforates, the nautilus-like *Polystomella crispa*; Schaudinn, for the latter alone. These organisms exist in two modifications, called macrospheric and microspheric respectively, according to the character of the central chamber (Plate VII.). The macrospheric is the sexual form. When the animal consists of two chambers only it contains both a nucleus and a chromidial mass (Plate VII., Fig. *c*). The grown animal when decalcified and stained is seen to be packed with small nuclei, which wander into the protoplasmic mantle to form the nuclei of the bi-flagellate gametes, or zoospores. When broods of the latter originating from different parent-cells meet they conjugate in pairs.

From the foregoing examples it will be seen that chromidial processes, which have been shown in Chapter IV. to play such an important part in the life of the mycetozoon *Didymium difforme*, occur widely among the Rhizopoda or Sarcodina, that great class of protozoa to which the amœbæ and mycetozoa belong.

CORRECTION.

In the description of Plate VII., Fig. "d" should have been referred to Schaudinn. All the figures in this plate were copied from Doflein's text-book, 2nd edition, 1909.

PLATE VII



STAGES IN THE LIFE OF POLYSTOMELLA CRISPA. *a*, Microspheric, asexual form, showing formation of merozoits; *b*, Merozoits separating as amœbulæ; *c*, Young macrospheric form showing nucleus and chromidium; *d*, Formation of microgametes. After J. J. Lister.

CHAPTER VII

RHINOSPORIDIUM KINEALYI (MINCHIN AND FANTHAM)

A Note on a Protozoon the presumable cause of Infective Papillomata of the Septum Nasi, the Conjunctiva, and the Penis.

The occurrence of any tumour caused by protozoa is of interest, and that described by Major O'Kinealy [1] in 1903 is particularly so in that the protozoa which it may be presumed are the cause of it have been examined and described by biologists,[2] as well as by pathologists.[3, 4] The latest paper which I have read is by T. S. Tirumurti.[5]

O'Kinealy's patient was a native of Bilhar. The growth which O'Kinealy removed at the Medical

[1] O'Kinealy, *Proc. Laryngological Society*, vol. x., 1903.

[2] Minchin and Fantham, *Quart. Journ. Micro. Soc.*, 1905–6, p. 52.

[3] Vaughan, report in O'Kinealy's paper referred to above.

[4] Beattie, *Journ. of Pathol. and Bacter.*, June, 1906, and *British Medical Journal*, December 1, 1906.

[5] T. S. Tirumurti, *Practitioner*, pp. 704–719, 1914.

College Hospital, Calcutta, was a papilloma of the size and shape of a large pea, attached by a peduncle to the mucous membrane at the anterior and upper part of the cartilaginous septum nasi; it projected into the vestibule of the left nasal fossa. The tumour bled frequently. It was easily and apparently completely removed by forceps and the cold snare, but it recurred after three weeks.

Major O'Kinealy, after reading his paper, kindly gave me a section of the growth; from this section the following observations were made. Vaughan's original description is verified in the microscopic appearance of the section as shown in Fig. 45.

Under a higher power, Plate VIII., the cysts, *a*, which were visible to the naked eye in the fresh tissue as white points, are seen to possess a central mass, which is chiefly composed of spherical or ovoid bodies which become larger from the outer to the inner part. Minchin and Fantham have described the larger of these bodies, which measure 5–6μ in diameter, as "spore-morulæ" containing from 9 to 15 refractile granules which they have found to constitute uninucleated "spores." The smallest, *i.e.* the most peripherally placed bodies, are uninucleated round or oval bodies, "pansporoblasts."

In Major O'Kinealy's section, which is

stained with picrocarmine and mounted in Farrant's

Fig. 45.—A Portion of the Section magnified × 60 diams. The presence in the epithelium and sub-epithelial connective tissues of rounded cysts with granular contents is the most striking feature. *a*, Connective tissue; *b*, Points to a collapsed empty cyst; *c*, The vacuolated part of the epithelium drawn on a larger scale in Fig. 45 *c*. × 60 diams.

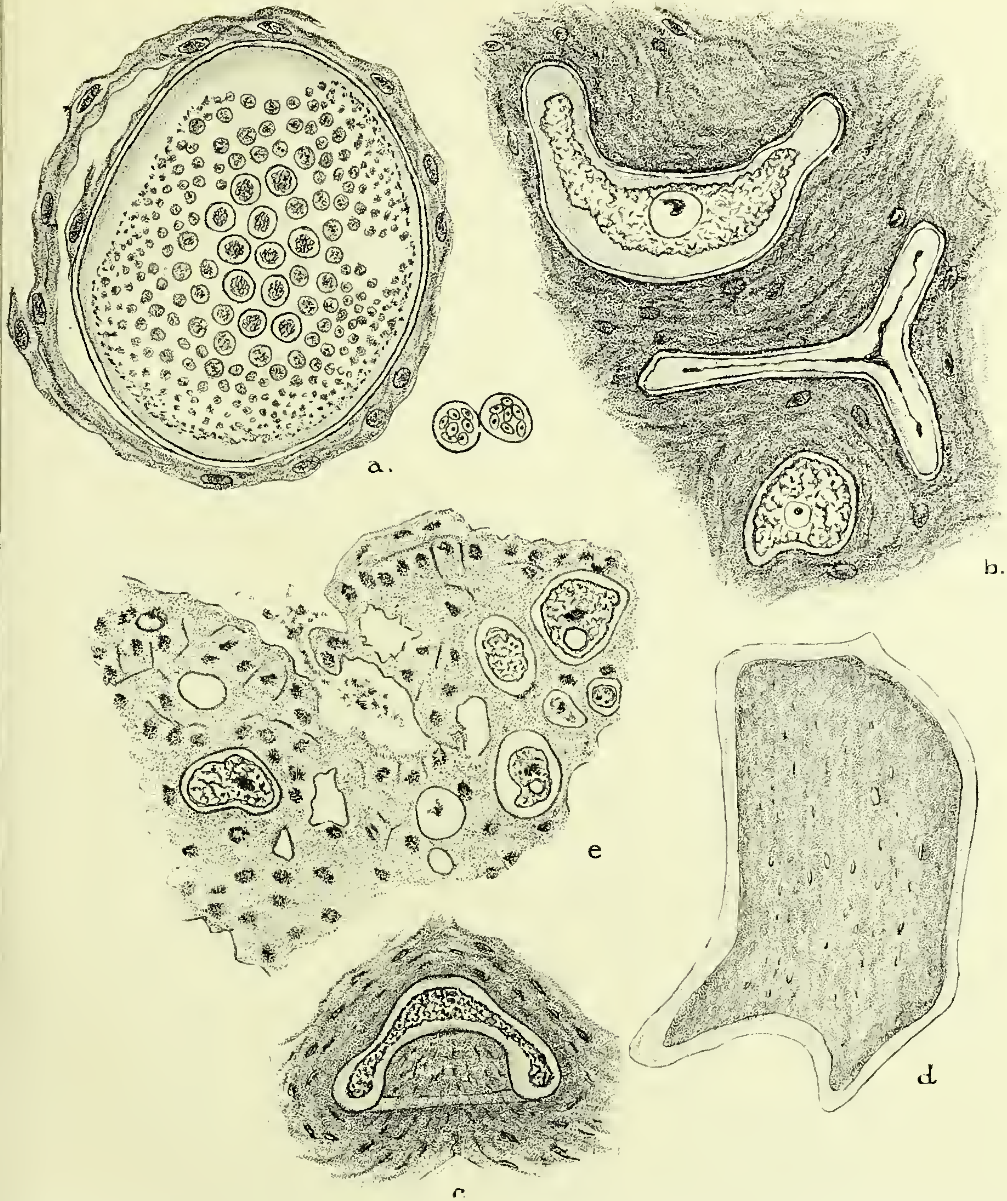
medium, the more mature spore-morulæ are stained

rather deeply with carmine, the rest of the granules being stained only faintly with picric acid. Minchin noticed the same feature in the specimen which he examined, and which, like mine, was prepared by Major O'Kinealy. Beattie worked on material supplied by Dr. T. M. Nair of Madras, who had met with four other cases similar to O'Kinealy's, all the patients coming from the small native state of Cochin, on the West Coast, and found that there was some support for O'Kinealy's impression that the cysts possessed a pore.

It is of interest to trace as much of the life-history of the parasites as is indicated in the section. The latter is necessarily of a thickness greater than the width of the largest cysts, which measure ·27 mm. in diameter.[1] In these largest cysts Beattie found that the spore-morulæ are separated from one another by a reticular structure. The youngest encysted parasites that can be seen in the connective tissue have the appearance shown in Plate VIII., *b*—a reticular body surrounded by a thin capsule and having a vesicular nucleus; above that is the remains of an empty cyst, and above that again a nucleated crescent-shaped

[1] The measurement of the cysts given by Vaughan are from ·144 mm. to 2·24 mm., and by Beattie 6 to 8 mm., the latter being more than twenty times as large as the largest seen in O'Kinealy's preparation.

PLATE VIII



RHINOSPORIDIUM. *a*, Oval cyst with a true capsule and an outer false capsule of fibrous tissue; below and to the right two spore-morulæ; *b*, Part of the tumour containing two nucleated parasites and a collapsed empty capsule; *c*, A dome-shaped non-nucleated parasite; *d*, A large non-nucleated parasite (?) in the chromidial condition; *e*, A portion of the epithelial covering (*c*, in Fig. 45) showing nucleated parasites with vacuoles between the epithelial cells. × 800 diams. The two spore-morulæ × about 1200 diams.

parasite with a capsule, hyaline cortical, and reticular central part.

From the study of one section it is impossible to say whether all the parasites pass through this cup-shaped phase, but in some, *e.g.* in Plate VIII., *c*, it is even more definite than in that shown in Plate VIII., *b*. At a later stage the parasites have an irregular form similar to that shown in Plate VIII., *d*, which also points to this parasite possibly having a chromidial phase. Such forms have been taken to indicate amœboid movement in the parasites. The possible presence of a pore in the fully developed cysts has been referred to above, and in regard to this, some of the parasites in which the reticular body forms at one point a rounded projection suggestive of a female receptive eminence which might well be associated with a pore, though I have not been able to detect one of the latter.

Beattie has described degenerated cysts and empty cyst-capsules invaded by granulation tissue. He has also sketched the fate of the spore-morulæ after they have been discharged from the cyst as follows: "Some are seen free in the tissues, and are usually surrounded by a mass of polymorpho-nuclear leucocytes, at places forming minute abscesses. No developmental stages could be traced. The spore-morulæ are undoubtedly discharged with the

secretions, but of their after history no clue was given in the specimens." The same author has found that phagocytosis occurs, but not extensively.

In the section on which this description is founded there were many spore-morulæ on the free surface of the epithelium together with blood-clot, etc. One large cyst is on the point of bursting on the surface of the tissue, having caused atrophy of the tissues covering it. In my section two morulæ and other bodies suggestive of developmental stages were present on the free surface of the tissue. In several places the epithelium has a vacuolated appearance, as in Plate VIII., *c*. On examining this with a higher power, reticulated amœba-like bodies, some with nucleus and vacuole, such as is shown in Plate VIII., *b*, were seen. They strongly suggest that the parasites hatch out from spores on the surface of the epithelium as amœbæ, penetrate between the epithelial cells, and thus reach the deeper tissue.

These appearances suggest the probability of the amœba-like bodies being stages in the life-cycle of the parasite, and at the present time, when our knowledge of the protozoa calls for enlargement urgently, it is desirable that the complete life-history of any protozoon be fully worked out before we can regard its classification as anything more

than provisional. It is to be hoped that further observations made on the living parasites will be forthcoming in order to complete our knowledge of the *Rhinosporidium Kinealyi*. Minchin and Fantham, in the article referred to above, do not critically consider the pathogenic *rôle* of the parasite, nor do they describe the nucleated amœba-like forms. Their statement that "the method by which the parasite succeeds in infecting fresh hosts must remain for the present a complete mystery," expresses the belief that the parasite causes the tumour. In this connection it should be remarked that the parasites are not so abundant or so closely associated with the tissue-cells of the tumour as is the case in molluscum contagiosum, or in cancer or sarcoma.

A sudden diminution of tension caused by rupture of cysts and the escape of their contents to the surface would account for the frequent hæmorrhages. It has yet to be proved whether or not papillomas of similar causation do not occur in this country.

Note.—All the figures accompanying the above account except the two spore-morulæ were drawn with the aid of a drawing eye-piece before the appearance of Minchin and Fantham's paper; guided by the latter, I was able to detect the spores in the morulæ.

CHAPTER VIII

CHORIOCARCINOMA

THE following notes appear to be best fitted to make a separate chapter, since they concern a particular variety of cancer. They confirm in no uncertain way the protozoan view of this disease. I have great pleasure in thanking Professor Alexander Primrose for permission to publish the results of my examination of sections he kindly sent at my request.

To introduce the subject I cannot do better than reproduce some of Primrose's original article,[1] and the illustrations (Figs. 46 and 47) accompanying it.

> "The patient, a married woman 30 years of age, had consulted a physician because of amenorrhœa. There was a history of menstruation having occurred when she was sixteen years of age, but on no other occasion. Examination determined the fact that the uterus was infantile; no further abnormality

[1] "Choriocarcinoma," by Alexander Primrose, *Annals of Surgery*, June, 1910.

was discovered. Three years subsequently she again consulted the same physician because of some vaginal discharge, and with the

Fig. 46.—Choriocarcinoma. Showing Langhan's cells and a few syncytial masses. (Microphotograph made in the Pathological Laboratory of Harvard Medical School from Primrose's case.)

belief at the time that she was menstruating. She had a slight amount of pain and discomfort. On examination it was found that the uterus was slightly enlarged beyond the normal size and was freely movable. She appeared to

be in perfect health, and there was no suspicion of any serious trouble. . . . The patient had been a strong active woman and up to the

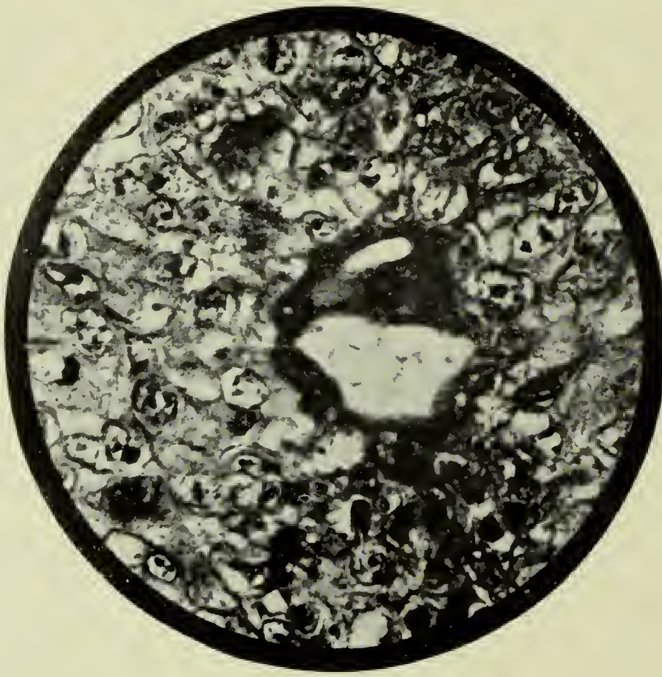


FIG. 47.—CHORIOCARCINOMA (HIGH POWER). Showing Langhan's cells. (Microphotograph made in the Pathological Laboratory of Harvard Medical School from Primrose's case.)

present juncture had exhibited no signs whatever of ill-health. Her mother died of cancer of the uterus at the age of 32.

Operation was undertaken for her relief. The tumour was found occupying the pelvis

and projecting considerably above the pelvic brim. The sigmoid flexure lay across its upper surface, upon which it was flattened out and firmly adherent. The tumour had infiltrated the sigmoid mesentery, and had somewhat extensively penetrated that structure and appeared upon its upper surface. On further examination it was found that the mass was firmly adherent in the lower portion of the pelvis and to the rectum posteriorly, whilst the uterus and bladder seemed to be embedded in it."

The pathological report received from Dr. O. R. Mabee was as follows :—

" The tissue received for examination consists of an irregular mass of soft deep red hemorrhage tissue which measures $10 \times 9 \times 7$ cm. On section a few grayish-white bands of tissue irregularly scattered are seen.

Histological Findings.—(Figs. 1 and 2). The tissues were preserved in formalin and Zenker's fluid. Sections were stained with eosin and methylene blue and Mallory's phosphotungstic and hæmatoxylin. The general histological appearance is that of a hemorrhagic tumour, scattered through which are solid masses of polyhedral epithelial cells with an occasional large multinucleated irregular deep-staining mass of protoplasm. The hemorrhages

occur widely distributed throughout the tissue. There is a fairly large number of vascular channels and an occasional lymph space which are walled by bands of the polyhedral cells. Irregularly scattered and closely related to the hemorrhagic areas mentioned above, are areas of degeneration and necrosis. Here the epithelial cells are crushed together, the nuclei are shrunken or have disappeared, and the cell outlines are indistinct. The tissues adjacent to these areas show a moderate amount of inflammatory reaction and a fairly large amount of granulation tissue.

"The polyhedral or Langhan's cells are well defined, and have a pale vesicular nucleus of relatively large size which is moderately rich in chromatin. The intranuclear network is well marked and the nucleus contains one or two nucleoli. Mitotic figures are fairly numerous. These cells generally occur in broad masses with little or no supporting connective tissue stroma. Scattered irregularly through these areas of Langhan's cells are seen occasional syncytial masses. This syncytium is not found in the form of branching buds, and there is the absence of villous formation. Teratomatous structures such as bone, hair, cartilage, etc., are also absent. Microscopical diagnosis, chorio-carcinoma (Ewing)."

In connection with these notes on chorio-

carcinoma, the extraordinary development of chromidia in the mycetozoon described in Chapters II., III., and IV. should be borne in mind.

A body similar to that shown in Fig. 44, *c*, is referred to in Dr. O. R. Mabee's pathological report embodied in Professor Primrose's article as a "Large multinucleated irregular deep-staining mass of protoplasm."

A portion of this choriocarcinoma drawn carefully with the help of a Leitz's drawing eye-piece is shown in Fig. 48. The arrow "a" points to a large body which lies near the middle of the drawing, and which I have described above as a chromidium, and which is shown drawn on a larger scale as Fig. 44, *c*. The arrow "b" in Fig. 48 points to a spiral body the lower end of which is globular. Structures somewhat similar occur frequently among the spirochætæ of syphilis, and the occurrence of spiral bodies in the case is worthy of notice since spirochætæ have been met with in human and in mouse-cancer, and in the infective sarcoma of dogs.

The arrow "c" in the same figure points to the dense intranuclear bodies usually considered to be nucleoli in malignant growths, and which are shown to be parasites by close critical study of the sections.

What is the origin of the protoplasmic masses such as are shown in Fig. 48 and in various

places in Plate IX.? By careful examination of the section under a 1-12th-inch oil objective some of them are found to have been on the point of escaping from

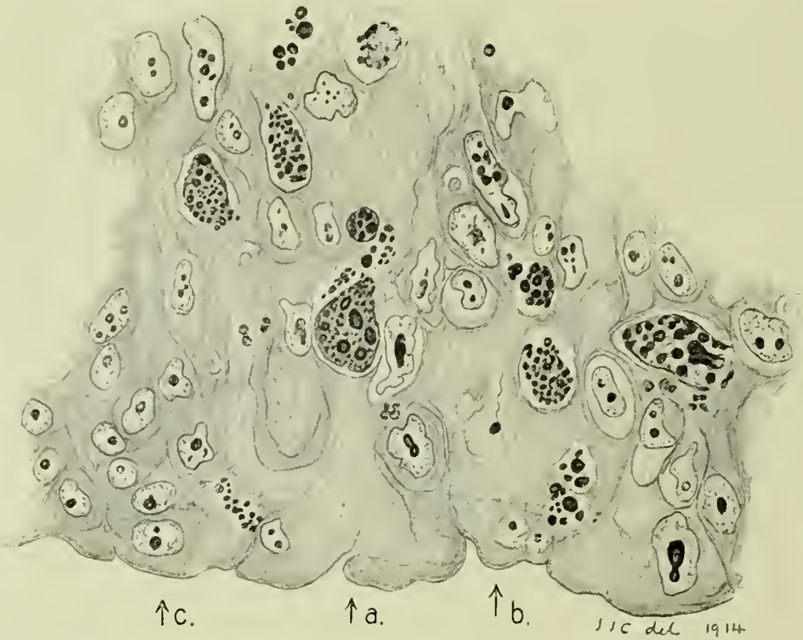


FIG. 48—A PART OF A SECTION SHOWING NUMEROUS CHROMIDIAL STRUCTURES. *a*, The chromidium shown on a larger scale as Fig. 44, *c*, Chapter VI.; *b*, A spiral body rising from a small chromidial sphere; *c*, Intranuclear chromidial parasite. × 800 diams.

nuclei, and evidently began their career as dense so-called nucleoli which escape into the cytoplasm, and thence into intracellular spaces. In this particular section many such bodies are stained a pale reddish

colour, and some of them show the vacuolated appearance (Plate IX., 2) often noticeable in their intranuclear stage also. This escape of pseudo-nucleoli to form the larger chromidial bodies is not so plainly seen in this choriocarcinoma as in that alveolar sarcoma of the breast which I described in 1913.[1]

At a later stage in these large free bodies chromatin appears in the form of strands or granules. Such granules may be peripherically arranged as in Plate IX., 3, or a central mass of chromatin and peripheral granules may both be present (Plate IX. 4).

A more definitely nuclear body may appear among chromidial granules as in Plate VIII., 5; or, as in 6, the chromatin particles may appear in groups resembling chromosomes during mitosis; or, again, they may be widely separated as in 7. In Plate IX., 8 the spirochæte-like body already referred to in Fig. 48 is drawn on a larger scale together with two other structures, in which minute spheroids are contiguous to filaments; the latter contain round points of chromatin at regular intervals. The chromatin in some of the chromidia is filamentous, as in some of those shown in Plate IX., 9 and 10. In "9" spiral forms of the parasites are shown side by side

[1] J. J. C., *Centralblatt für Bakteriologie*, 1893.

with globular and irregular forms and two empty nuclear membranes and some cytoplasmic remains. In "10" tumour cells and red blood corpuscles are shown together with filamentous subdivisions of a parasite containing chromatic filaments and three small ovoid bodies in which no chromatin has separated.

In this choriocarcinoma I have found a definite structure which can hardly be anything save an amœboid protozoon (Plate IX., 11). It approaches the Leydenia form of *Chlamydophrys stercorea* as described by Schaudinn and of which Doflein (Lehrb., p. 584) has written that it is the first body to be established as a protozoon by an eminent protozoologist in a condition declared to be cancerous by a clinical authority. The body I now describe lies within an intercellular space in the tumour with some blood-cells. Its central part has the same character as the chromidia I have described above; its periphery consists of expansions containing minute but definite chromatic particles and terminating in fine pseudopodia.

Other protoplasmic masses or chromidia I found to be breaking up into stellate subdivisions, as for instance in Plate IX., 12, similar to but smaller than those I have previously described in the sarcoma of the breast referred to above, and of which this

PLATE IX

VARIOUS STRUCTURES IN THE CHORIOCARCINOMA, details in text.
× 800 diams.

choriocarcinoma recalls another feature :—the possession of knobbed processes by some of the intranuclear bodies (Plate IX., 13). Many years ago, similar structures led me to suspect that the parasites in the sarcoma of the breast might be suctoria, but prolonged study has led me to recognise them as belonging to the sarcodina, though many points in their life-history remain to be cleared up.

Not all the intranuclear parasites in this choriocarcinoma escape from the nucleus before they undergo further change : in many of them further development occurs whilst they are still within the nuclear membrane. In some instances (Plate IX., 15, 16, 17), I found that they give off peripheral extensions which subdivide into stellate amœbulæ which fill the nuclear membrane and pass beyond it, the nucleus and the whole cell becoming greatly enlarged. This mode of formation of amœbulæ, like that referred to above within the small blood-vessels, recalls the mode of subdivision seen in some of the Foraminifera, for instance, *Polystomella crispa* (see Chapter VI., p. 104).

It remains now to add a few words on the mitotic figures seen in their section. They differ from those of normal human cells that I am familiar with, the regular stages of such mitosis being absent : there is, for example, no spireme stage, and the

chromosomes have polygonal outlines, and their mode of origin is different, as Plate IX., 15 shows: here chromosomes are being formed by the loosening of a previously dense intranuclear body. The mitotic figure is complete with centrosomes in Plate IX., 19. In the same figure Nos. 20 and 21 show mitoses in cells in which there are within the cytoplasm numerous particles which stain darker than the chromosomes, black as compared with blue.

The mode of origin of the chromosomes in these mitoses and their polygonal form confirm me in the opinion that this belongs to parasites as I have previously stated of similar mitoses in other malignant growths.

The mitoses such as those just referred to may ultimately prove to be reduction mitoses preliminary to the formation of sexual elements.

Many important details, which are not shown by even the best micro-photographs, become clear on making careful drawings: a comparison of the illustrations in the present chapter proves this point fully.

CHAPTER IX

THE POSITION OF CANCER IN PATHOLOGY

In order to see how the position of the pathogenic protozoa stands at the present time in text-books of pathology I turn over the leaves of the most recent,

Fig. 49.—Bird's-eye Bodies in Cancer of the Breast. After F. B. Mallory.

and in my opinion the best, work on the structure of diseased tissues in the English language: F. B. Mallory's "Pathologic Histology," 1914. In the chapter dealing with the general pathology on page 254 is a photograph, the essential part of which is here copied (Fig. 49). The legend beneath the figure

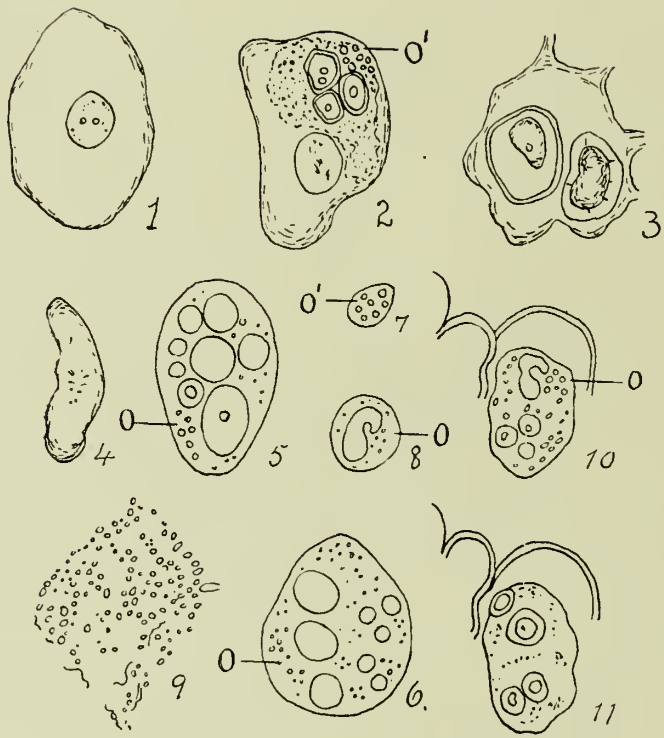


Fig. 50.—Bodies observed in a Warm-Stage Preparation.

From the author's article in the *Transactions of the Royal Medico-Chirurgical Society*, 1907.

Fig. 50.—Bodies observed in a Scraping of a Chronic Specific Epithelial Growth. (Examined on the warm stage under a 1-12th inch oil-immersion lens.)

1, An ordinary squamous epithelial cell devoid of movement.

2, A similar cell, but containing in optical section a granular mass in which are three typical bird's-eye bodies, and at *o′* a group of granules in lively oscillation.

3, Another epithelial cell, the nucleus of which is not seen, and which contains two highly refracting inclusions.

4, A free body of similar optical characters to the inclusions in 3.

5 and 6, Structures resembling an epithelial cell in size, but containing large globules of a bright greenish appearance, and at *o* and *o′* groups of smaller globules in oscillation.

7 and 8, Small bodies resembling parts of 5 and 6, lively oscillation in one, and oscillation and a greenish nuclear body in the other.

9, A large body, the pale central mass of which is not shown, but globules and granules and a few wavy lines are seen on the surface; (?) involution forms.

10 and 11 are the same body, 10 as seen at 7.20 p.m. and 11 as seen at 10.45 p.m., when the preparation has cooled down. When first seen, there was a group of oscillating granules, *o*, and a single greenish curved structure to the left of them; later, the preparation having cooled down, the oscillating granules had disappeared, and the body was seen to have divided into two.

reads: " Carcinoma of mammary gland. The hyaline bodies are products of secretion or degeneration, and were formerly exploited as parasites and the cause of cancer." Thus it appears that pathologists are still unable to say what these bodies are, and at the same time they do not hesitate to say what they are not, and this " not " is an immense one, seeing that it includes all the myriad forms and phases of parasitic protozoa, known and unknown. The bodies in question are best named " bird's-eye " bodies. They are among the structures designated by Virchow in 1847 as " cells of endogenous origin," with the taint of untested assumption that they were " perhaps " nuclei which " had fallen out." They are the bodies which H. G. Plimmer first considered to be the only form of protozoa to be found in cancer, and later, having isolated a common blastomycete from a cancer, declared the same bodies to be blastomycetes. That they really are protozoa formed from chromidial masses can be proved by any observer who will take the trouble to examine patiently scrapings of cancer or precancerous syphilitic lesions on a warm stage (see Fig. 50). They are formed by free-cell formation from chromidial bodies, which can only be protozoa.

These bird's-eye bodies are the least constant and the least important of all the various phases of

protozoa to be found in cancer and other diseased tissues.

By limiting their attention to this one phase of the protozoa of cancer, pathologists overlook both the earlier intranuclear phases such as are shown in

FIG. 51.—INTRANUCLEAR, INTRACYTOPLASMIC, AND FREE PARASITES IN CANCER OF THE BREAST. Drawing eye-piece. × 800 diams.

Fig. 51, and the later ones equivalent to those shown in Figs. 52, 53.

Phagocytosis in Cancer.—Some critical notice is demanded for the phenomena of phagocytosis as observed in certain cancers. Fig. 52 shows part of the squamous-celled cancer which has already been referred to in Chapter III. There are three

hyaline bodies which are officially known in London as "keratinosed epithelial cells." Above the largest of these is a similar body subdivided into segments analogous to those regularly observed in cultures of the

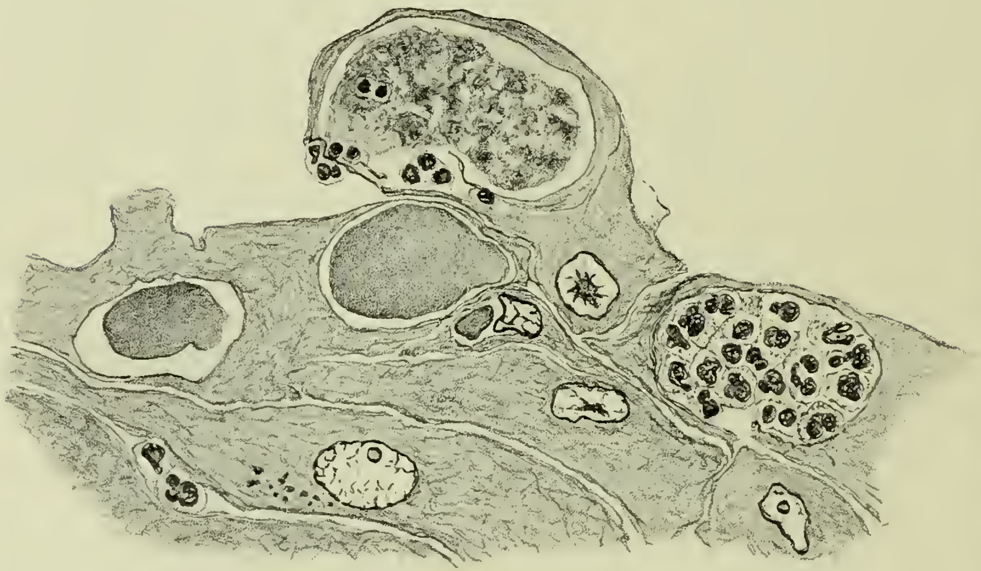


FIG. 52.—CANCER OF THE SEPTUM OF THE NOSE. Epithelial cells, three chromidial protozoa, one segmented protozoon, and leucocytes. Drawing eye-piece. × 800 diams.

protozoa of molluscum (see Plate IV.). In the substance of the segmented body is a common leucocyte, four others being close to the segmented body. To the right of the figure is a space completely filled by leucocytes. Close critical study of the cancer in which this body occurs, and similar tumours, shows

that the hyaline bodies are protozoa in the chromidial state. They only become chemiotactic, *i.e.* attractive to leucocytes, when they subdivide into segments. It is, of course, not difficult to find portions of cancers

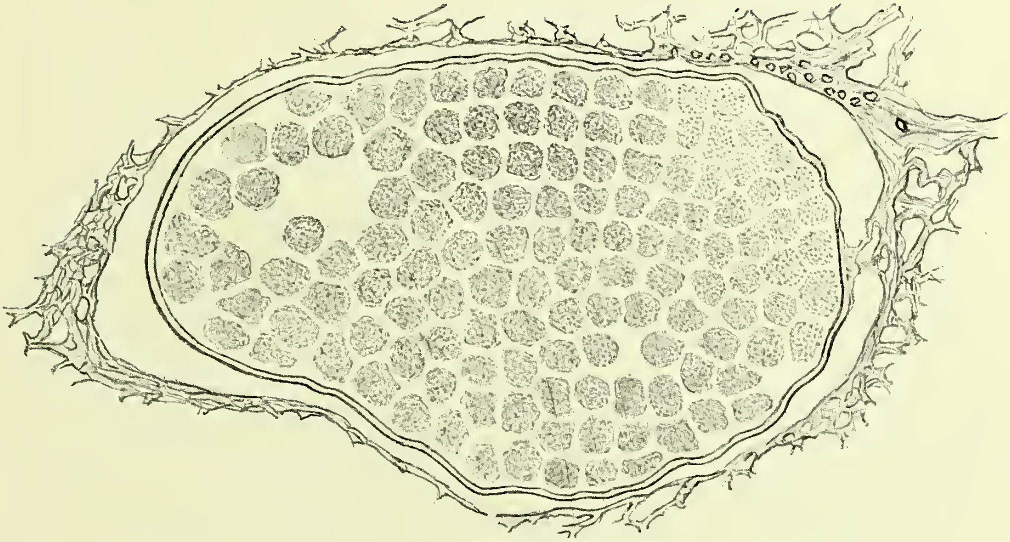


Fig. 53.—Cancer of the Nasal Septum. An encapsuled protozoon subdivided into granular "spores;" at one point the double-contoured capsule is continuous, with a supporting framework exactly like that of the mycetozoon *Didymium difforme.* Drawing eye-piece. × 800 diams.

in which the subdivided parasites have all been replaced by leucocytes, and such have been advanced as evidence against the interpretation here given of objective documents, such as Fig. 52. Such reasoning is, it need hardly be insisted on, quite unsound.

Another striking structure from the same tumour is worth careful study (Fig. 53). Within a perfect capsule which at one point is connected with a supporting framework exactly like that of the mycetozoon described in Chapters II., III., and IV., are seen a great number of granular bodies. This is a farther stage of the fragmentation the beginning of which is seen in Fig. 52. There are no leucocytes present, a fact which is explained by the complete encapsulation of the subdivided mycetozoon. Here, in order to give due emphasis to a very important matter, attention may again be called to that other element from the same cancer already figured in Chapter IV., Fig. 27, and it must be borne in mind that these are not accidental growths of mycetozoa on an ulcerated surface, but are links in a series of forms which can be traced by their optical characters as beginning as minute intranuclear bodies, and which are essential features of every spontaneous cancer.

It is inevitable that such late stages of the parasites should be met with where they have access to air and light, which are necessary to the corresponding stages of free-living mycetozoa. In such positions they are easily detached and so not seen in sections.

Only many decades of Virchow-hypnosis can explain the obliquity of vision which leads to objects such as are shown in Figs. 27, 52, and 53 being

persistently pronounced by those who speak officially for pathology in London to be "keratinosed" epithelial cells.

Among many objects in cancer and other diseased tissues wrongly interpreted as phagocytos,

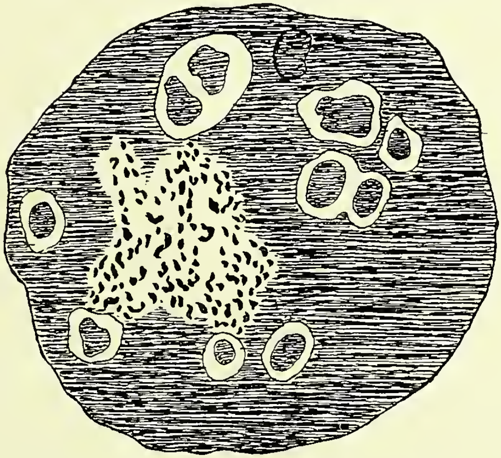


Fig. 54.—"Phagocytosis in a Large Epithelial Cell of a Squamous-celled Epithelioma of the Lower Jaw." Jos Claessen, Ziegler's *Beiträge zür Pathol. Anat.*, Bd. XIV. Heft I., 1893.

the one shown in Fig. 54 may be instanced. This was described as "Phagocytosis in a large cell of a squamous epithelioma of the lower jaw." In the opinion of the pathologist who described it we are to believe this to be an epithelial cell which has had a good meal. Now, in my experience it is rare, if not unknown, for a human epithelial cell to act as a

phagocyte. And the nucleus is a strange one for a cell of a squamous epithelium. Then the nature of its meal. The bodies inside the cell cannot be leucocytes, because there is no trace within them of a nucleus, which would be the last part to resist digestion. Large cells like the one under consideration are common in rapidly growing sarcomas; in them careful comparison with surrounding cells shows that the nucleus is chromatin differentiated from a previously undifferentiated protozoon in the chromidial state, and the contained bodies are merozoites, again in the chromidial state.

The pathologist's outlook.—This may be judged by the way in which tumours are regarded in contemporary works. As far as I can see classification still engrosses pathologists, to the exclusion of causation. It should be remembered that this classification is based on study of sections under low magnification, and ignores crucial facts.

In a recent article,[1] T. T. O'Farrell has put into form the Virchow-Cohnheim classification of tumours as applied to those of the kidney especially. He writes:

"I proceed to recapitulate briefly the general

[1] Thomas T. O'Farrell, "Adenocarcinoma (Mesothelioma) of the Kidney," *Medical Press and Circular*, June 23, 1915.

types of tumour to be met in the kidney, with special reference to Adami's classification, which is based on embryological considerations. For the sake of lucidity I have drawn up the main headings in the form of a table, my object being to tentatively place this particular tumour in its proper position in the list."

Classification of Tumours (Adami).

(Modified with special reference to kidney tumours.)

I. Teratoma. Derived from topipotential cells.

(i.) Twin Teratoma: Fœtal inclusion.

(ii.) Filial Teratoma: Separation of embryonic totipotential cell with subsequent growth.

Kidney dermoid very rare.

II. Teratoblastoma. Derived from pluripotential cells.

(i.) Diphylic: containing derivatives of two germinal layers; rare kidney tumours.

(ii.) Monophylic: Containing derivatives of one germinal layer; most mixed kidney tumours.

III. Blastoma. Derived from unipotential cells.

(i.) Heterochthonous: Cells derived from another individual.

(ii.) Autochthonous: Cells derived from own individual unipotential cell (ordinary tumours).

Autochthonous Blastomas (ordinary tumours).

A. Lepidic, or Rind Tumours (cells grouped together, groups not penetrated by blood-vessels, and no definite intercellular substance).

(i.) *First Order* (derived in direct descent from epi- or hypoblast).	(1) Epiblastic origin. (2) Hypoblastic origin.
(ii.) *Second Order* (derived in indirect descent through a mesoblastic stage, hence—Transitional).	(3) Mesothelial (*a*) typical adenoma of kidney; (*b*) atypical, carcinoma of kidney and hypernephroma. (4) Endothelial.

B. Hylic, or Pulp Tumours (cells separated by intracellular substance in which blood-vessels may be found).

(i.) Epiblastic.
(ii.) Hypoblastic (chondroma).
(iii.) Mesenchymal:
  (1) Mesenchymal: (*a*) typical: fibroma, lipoma, etc.; (*b*) atypical: sarcoma.
  (2) Mesothelial : rhabdomyoma.

No one recognises more than the present writer the value of the work of those who have established the rudiments of the histology of cancer, sarcoma, and other tumours; that in the majority of such tumours a basis of one or another tissue can be recognised is now a truism, and the fact is of daily, almost hourly, service in diagnosis and to some extent in prognosis and treatment, but as far at least as the common spontaneous cancers and sarcomas are concerned, it is a fact from which false inferences have been drawn. One false inference is that the elements derived from the victim's body are the only obvious cells to be recognised easily under the microscope,

the higher powers of which should be used as a routine in examining sections of tumours. Classifications of tumours such as that given above represent a Linnæan analysis, the infinite variety is recognised and dealt with, and it may be added that there is the Linnæan habit among pathologists of referring anything that does not fall in with their grouping as "chaos." The Darwinian view, the inquiry into the cause of all this variety, has not seriously occupied the mind of official Pathology. Lest this statement should be doing an injustice, I will examine briefly a recent account of some tumours emanating from the distinguished pathologist[1] who may be regarded as the official representative of pathology in London. The paper is entitled "Eleidoma," and it deals with certain tumours in man and fowls marked by the formation of horn-like masses. A careful reading fails to disclose in the whole article any suggestion that any cause for the gross changes in the tissues has been in the writer's mind. The conclusion shows the writer's attitude of mind :—

> "I have ventured to name the tumour in question an 'Eleidoma,' after Virchow's manner of using the term 'Psammoma'; for although the latter is only an endothelioma,

[1] S. G. Shattock, "Eleidoma," *Proc. Roy. Soc. Med.*, 1913–14, Path. Sec. pp. 119–139.

it is one in which a striking amount of calcification takes place in the endothelial whorls; and the master thought this of sufficient importance to justify the use of a distinctive name."

There is a soothing quality about this paper on Eleidoma: it is like listening to some favourite melody, some well-loved hymn even. It would seem sacrilege to introduce any new harmony into the music. But there is danger even in singing favourite hymns. If we keep on singing "Peace, perfect Peace" when the Enemy is at the gate, we are risking annihilation. And if we continue for ever believing and repeating the Master's error: "All that lives in cancer is nucleated cells," when protozoa in the shape of chromidial particles are invading the most vital parts of the cells of countless children as well as one in ten of adults who are sick unto death, we are doing our best to ensure the continuation of the annual slaughter that cancer effects.

The view of cancer at present accepted, namely, that it is due to a rebellion of our own cells against us, is the least likely of any to be correct. Shutting our eyes to the protozoa in cancer may be compared with what would prevail in this country at the present time if the Press and all who have the ear of the people persisted in ignoring the existence

of hostile armies and fleets of the air and the under-sea; then all the loss of the British and allied nations would be ascribed to a hideous epidemic of suicide and self-mutilation.

As a statement of the view of the nature of cancer at present held by pathologists, I may again quote from the work of F. B. Mallory, p. 253:—

> "In the past few years, however, injury, severe or long continued, has come to the front again as a frequent cause of certain types of cancer, and possibly of some other varieties of tumours. This change of view is due to the repeated occurrence of carcinoma following excessive exposure to X-rays and radium. A similar formation of carcinoma following exposure to ordinary sunlight occurs rarely in a very few susceptible individuals (melanoderma). The exact manner in which the cancer arises has not been fully determined, but it seems to be due, not to direct stimulation of the epithelium, but injury done the connective tissue and blood-vessels, as a result of which excessive regenerative efforts on the part of the epithelium are called forth"

Now, with regard to this view we may recall the fact that certain cases of sarcoma have been cured by the therapeutic use of X-rays. It is possible that a certain dosage of a violent and penetrating

radiation regularly repeated day by day, is the nearest approach to the effect of parasitic protozoa extending

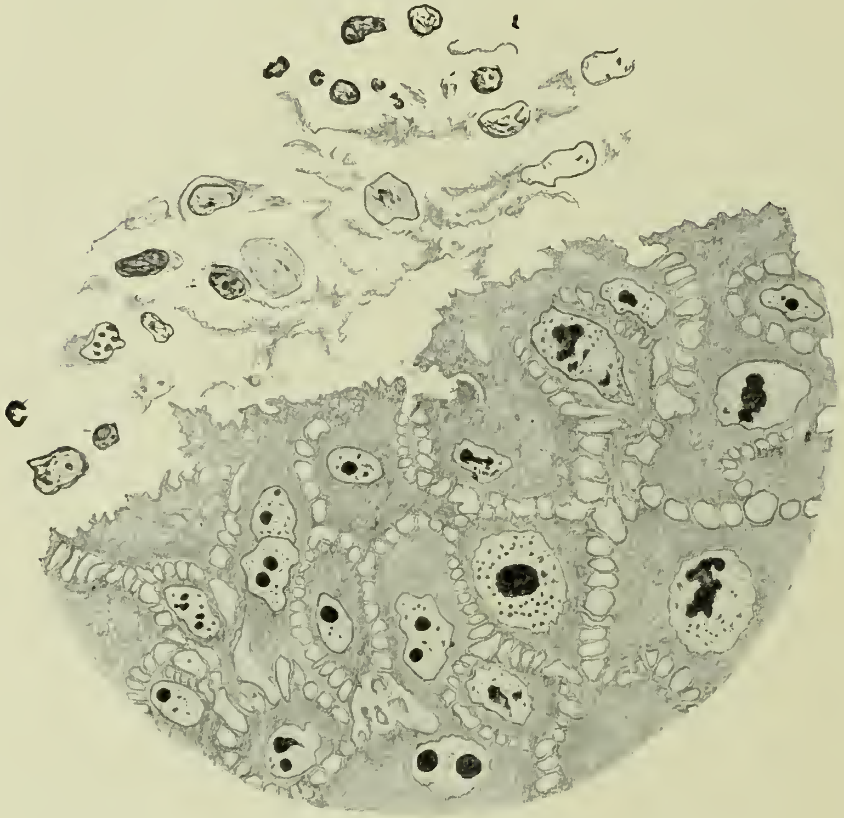


Fig. 55.—X-ray Cancer. Below, normal epithelial cells; above, degenerated connective-tissue cells. × 800 diams.

cyclically in the tissues. It is worth while to examine X-ray cancer very minutely to see whether the bodies I describe as the protozoa of cancer are present in and among the cells. In Fig. 55 is shown the minute

structure of one of the deep epithelial extensions in a case of X-ray cancer of the hand. There are none of the protozoa in it, and this is what is to be expected. Fig. 56 shows another part of the same section: here

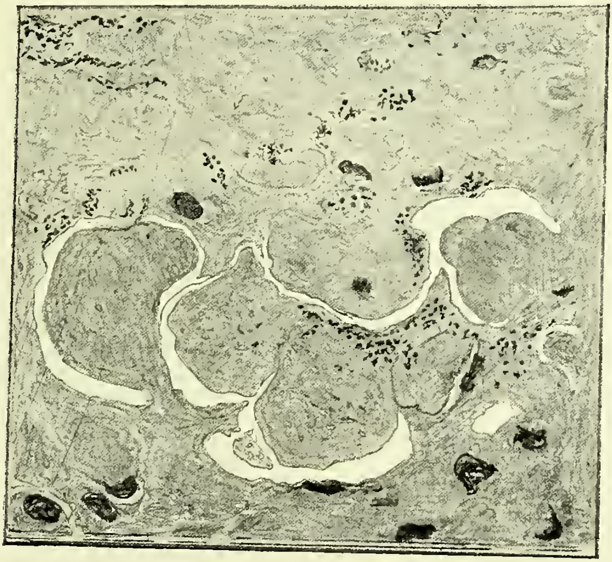


FIG. 56.—X-RAY CANCER. Degenerated and swollen cells ot the superficial layers of the skin; a true hyaline degeneration which may deserve the designation "keratinosed." × 800 diams.

hyaline bodies are seen which at first glance might be thought to be the same as the larger chromidial protozoa of squamous epithelioma, but the briefest critical examination proves them to be quite different. If ordinary irritation caused cancer, every corn would

become a cancer, and every one who shaves would develop cancer. With regard to special irritants, such as soot or tar, the position is different; these materials contain substances which are said to foster the growth of certain protozoa. But there is no end to armchair cogitations, and they are unnecessary; any one who will take the trouble to study a common mycetozoon until he or she is familiar with its chief aspects under the microscope, and then study any typical spontaneous cancer or sarcoma with critical and unprejudiced eyes will soon realise that the protozoa are there, and that in such numbers and in such relation to the cells and tissues of the host that they account fully for the tumour and any extensions it may have.

CHAPTER X

"ULTRA-MICROSCOPIC" CAUSES OF DISEASE, "CHLAMYDOZOA"

SINCE 1898, when it was proved that the virus of pleuropneumonia of cattle[1] could pass through a Berkefeld filter, a considerable list of diseases in which the virus is filterable have been compiled. Among them is *Molluscum contagiosum.* It has been shown above in Chapter V. that these bodies which are far from being ultra-microscopic, are protozoa belonging to the mycetozoa group. It is not to be doubted that the same will prove true in other diseases now thought to depend upon ultra-microscopic organisms, if the latter are given an opportunity of exhibiting vital processes. From personal observations I believe the Guarnieri bodies to be protozoa, and they, together with the kindred bodies of rabies and of trachoma, may be briefly considered here. It has been too readily assumed that to pass a fine porcelain filter, even the finest, that known as the

[1] Nocard, Borrel, and Roux, "Annals of the Pasteur Inst.," 1898.

Chamberland "B," a micro-organism must necessarily be ultra-microscopic. Now, given time, an organism as large as an elephant could pass through such a filter, if it could assume a linear shape and had a tenuous viscous character. I have no doubt that other viruses now thought to be ultra-microscopic in time will be proved to have phases in which they can almost be recognised by the naked eye.

Bird molluscum.—This disease has a virus which is filterable, and after being so filtered the virus is very resistent to hostile influences. Much has been written about it. The flagellate "diphtheria" of birds is probably the same infection. Et. Burnet[1] found that inoculation of the cornea with bird molluscum was followed by the formation of cell-inclusions like Guarnieri's bodies in small-pox and vaccinia. Schuberg and Schubotz,[2] who apparently made no cultivation experiments, concluded that the viscous character and partial solubility, even in water, opposed a valid reason against their being protozoa. On the contrary, the observation recorded in Chapter II. of the miscibility of the mycetozoon, Didymium, with water, and the viscous character of the

[1] Et. Burnet, "Annals of the Pasteur Inst.," September 25, 1906.

[2] A. Schuberg and H. Schubotz, *Cent. f. Bakt.*, October, 1910.

chromidial "pools" of this protozoon and the same feature of the protozoa of cystic ureteritis prove their contention to be ill-founded. The apparent miscibility with water is really a multiple minute plastotomy, and it explains the filterability of the virus, but does not prove the bodies not to be a protozoa.

Small-pox and vaccinia.—When all the history of this subject is written, I think it will be

Fig. 57.—Bodies seen on the Warm Stage in Vaccine Lymph. One is stellate and amœboid, and appears to be breaking up into minute subdivisions—its ultra-microscopic phases. After Lionel Beale, 1864. × 1200 diams.

recognised that an Englishman, Lionel Beale, was the first to describe the protozoon of vaccinia. In 1864 Beale[1] described the bodies shown in Fig. 57. The original description reads—"Germinal or living matter from a vaccine vesicle showing changes in form which occurred a few minutes after it was transferred to a warm stage." In 1893 Guarnieri published the results of inoculation of the cornea of rodents with vaccine and with variola lymph, being

[1] Lionel Beale, *Quart. Journ. Microsc. Science*, 1860.

led to make the experiments by the observations of Van der Loeff and L. Pfeiffer. In 1893 I was able to confirm these results, and in 1895 I published an extension of them. The appearance of Guarnieri's bodies in the inoculated cornea is so constant that it can be used in diagnosis in doubtful cases of variola. Calkins, Councilman, and Lafleur discovered the intranuclear phases which distinguish the variola double cycle from the single one of vaccinia. No one has worked more diligently at this and related subjects than F. J. Bosc,[1] from whose article on "The Parasite of Small-pox, its Schizogonic and Sporogonic Forms" some illustrations will be given here. An abstract of Bosc's observations will serve to form a descriptive account of Fig. 58.

The Protozoa of small-pox.—The protozoon of small-pox presents itself in two cycles of evolution; one passes through its phases in the protoplasm, the other in the nuclei of the epithelial cells of the pustule. *Intracytoplasmic forms:* these exactly resemble the forms that occur in vaccinia. The smallest granules are both basiphile and oxyphile, being in the chromidial state. Around some a zone of protoplasm develops, chromatin in the shape of fine granules appearing in the protoplasm; the process ends by segmentation of the protoplasm, merozoit

[1] F. J. Bosc, *C. R. Soc. de Biol.*, October 30, 1903.

formation. In small-pox there is also a formation of chromatozoites.

Intranuclear forms.—The early intranuclear forms are granules with a clear centre; these grains become larger (5–6μ) and consist of a central

Fig. 58.—Various Forms of the Parasite of Small-pox. 1 to 10, intracytoplasmic stages; 11 to 23, intranuclear forms. All the bodies here depicted, except the remains of the epithelial cells, are still regarded in official pathology as leucocytes! After F. J Bosc.

corpuscle and a more voluminous body. This form becomes subdivided first into several, later into many (2–4μ) bodies formed of a delicate capsule surrounding a central corpuscle arranged in a morula, without a general capsule. They finally break through the remains of the nuclear membrane. Sometimes the nuclear matter of the parasite divides before the

protoplasm, giving rise to a number of small bodies enclosed in a general capsule. Another and abundant form consists of a voluminous protoplasmic centre enclosing two central corpuscles, and surrounded by a series of vesicles of variable size, the whole enclosed in a thick capsule. The nuclear corpuscles in the central protoplasm divide, forming several multi-nucleated encapsuled bodies, which again divide to form a great number of nucleated vesicles (2 to 4μ); these burst the nuclear membrane of the host-cell and escape into a large intra-cytoplasmic vacuole, leaving a residual body. These bodies may also develop in the cytoplasm. The resulting minute vesicular bodies are very abundant, being either massed together in the protoplasm of the degenerated cells or free in the liquid of the vesicle. Mann's eosine methyl blue colours them well.

Neuroryctes.

The term "Neuroryctes," or the "nerve-cell-breaker" (Fig. 59), has been given to the corpuscles first described in 1903 by Négri[1] in the cells of the central nervous system of animals killed whilst

[1] A Négri, "Contributo allo studio dell' eziologia della rabbia," *Boll. d. Soc. Med. chir. di Pavia*, vol. iii., 1903. Also, E. Bertarelli, *Cent. für Bakt.*, Abt. I., p. 556, 1906, where the literature up to that date is given.

suffering from hydrophobia. The term implies that these corpuscles represent a stage of development of a parasite which is the cause of the symptoms of the

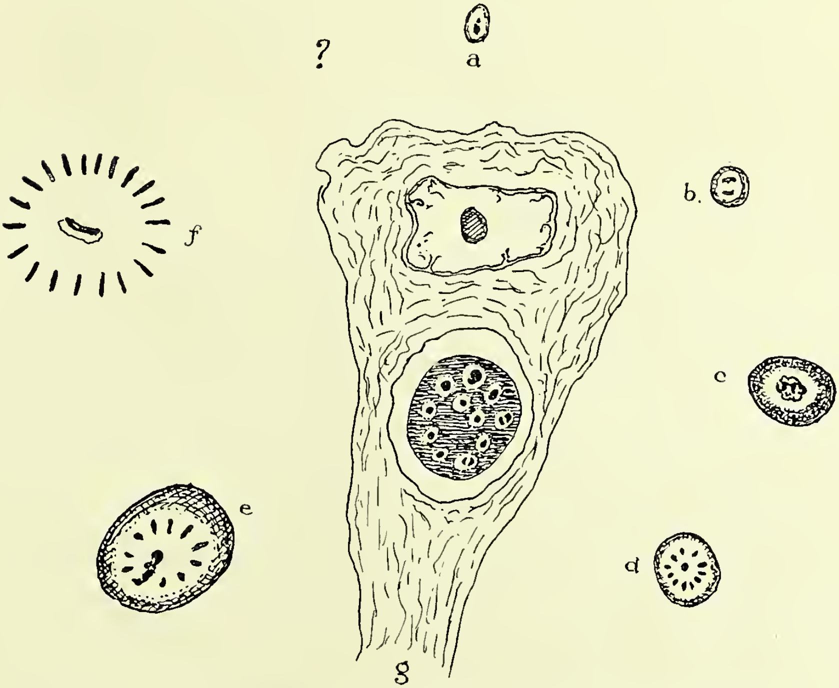


Fig. 59.—Hydrophobia : Negri's Corpuscles. *a—f*, Series of stages ending in a radial arrangement with residual body ; *g*, Intracellular stage ; (?) Filterable stage. Diagram based on Volpino's drawings.

disease, the symptoms and lethal termination of which are caused by its destructive action on the cells of the brain and spinal cord. The corpuscles are most easily seen in the cornu ammonis

(*Hippocampus major*), though they are present also in the cortex of the brain and in the spinal ganglia.

The opposite view, to the effect that these Négri corpuscles cannot be parasites, has some supporters, who base their opinion on the fact that the virus of the disease has been found to be capable of passing through a porcelain filter.

An important diagnostic test is based upon the undoubted fact that these bodies are readily recognised in preparations made by simply pressing cover-glasses upon the cut surface of the *Hippocampus major* of a dog that has been killed when suffering from hydrophobia.

The corpuscles are seen in the body (cytoplasm) of the pyramidal cells. They can be seen in the fresh unstained preparation simply mounted in saline solution. In preparations fixed and stained with Mann's eosin-methylene blue method, the bodies assume a red colour which contrasts with the blue-stained cytoplasm of the cell.

The bodies vary in number and in position in the cell, where they may lie near the nucleus, or at some distance from it, even in one of the prolongations of the cell.

There is no need to dwell upon the value of this simple diagnostic test when a person has been

bitten: in a few minutes we can decide whether it is necessary to send the patient to Paris to undergo Pasteur treatment. In our own island, thanks to Mr. Long's muzzling orders and to the quarantine regulations for dogs, this question is not likely to arise save in very few cases. On the Continent the question arises more frequently, and this method of diagnosis saves priceless time, and is more reliable, as well as more rapid, than the older one of examining the blood-vessels of the medulla for perivascular infiltration.

From this particular application of the knowledge Négri has given to the world, we may pass to consider in greater detail points of their structure. The diameter of the bodies varies from 1 to 20μ; even 26μ has been observed. Their form varies; they may be round, oval, spindle-shaped, or triangular. The small forms are linked to the larger by a gradation of sizes.

Though of variable shape, the bodies have a definite structure, containing corpuscles which may be arranged in a rosette-form.

Négri's bodies have been found in dogs, cats, cattle, rabbits, birds, and in man. They are smaller in the rabbit than in the dog, but their distribution is similar.

The work of Négri and other observers have

established the following data concerning Négri's corpuscles:—

1. They have been found in all but one or two per cent. of animals proved to have had rabies when killed.

2. They are plainest in the dog and the ox. They are larger in man than in the rabbit.

3. They appear when filtered virus is used to inoculate an animal.

4. They have not, up to the present, been found in the salivary glands or peripheral nerves.

5. They are resistent to glycerine, drying, and decomposition.

6. The *Cornu ammonis* is found to contain the virus four days before négri bodies appeared in controls.

Are they parasites, or but garments of a fashion new among protozoa?

(1) They are *specific*, as proved by their absence in the brain cells of animals killed by poisons; eosinophile granules in brain cells have not the same structure.

Volpino, by careful histological work, has found that they consist of—1, ground substance; 2, large corpuscles (inner formations), round or oval, 1–3 in number, placed near the middle of the body; 3, round, glistening small corpuscles (small inner

formations) lying near the periphery or scattered through the ground-substance. These inner bodies stain sometimes red, sometimes blue with Mann's stain.

Trachoma.—An English ophthalmologist, N. C. Ridley, of Leicester,[1] was, I believe, the first author to describe the cell-inclusions of trachoma as being of the same nature as molluscum bodies, and I

Fig. 60.—*a*, Epithelium from the Conjunctival Fornix in a case of Trachoma. Showing oval bodies. Zeiss E. = 1-8th inch. *b*, An oval body. After N. C. Ridley.

will give in this place a short quotation from the original paper with two of the illustrations (Fig. 60).

> "Now with regard to these globular bodies described as goblet-cells by Reid of Glasgow and by Mütermilch of Warsaw, certainly some of them may be goblet-cells, as those structures undoubtedly do occur in the normal conjunctival epithelium, especially in

[1] Ridley, *Transactions of the Ophthalmological Society*, vol. xiv., 1894.

the Krause's fold region. It would only be rational to expect that these would be increased in number in consequence of an inflammatory condition, and so hyperæmia of the subjacent part.

"But I venture to think that some of the bodies in trachoma cannot be accounted for in this way.

"Firstly, they are far too numerous.

"Secondly, some are found almost alone at a part where goblet-cells are not ordinarily found, and sometimes they are present in squamous epithelium.

"Thirdly, many are not in contact with the free edge of the epithelium as goblet-cells should be, but are in the deepest layers.

"Fourthly, many have their long axes not at right angles to the surface, as is the case with a goblet-cell, but in various directions, and in some cases even parallel with the surface.

"Fifthly, their reaction to staining fluids differs.

"On hardening a specimen with Müller's fluid or spirit, nothing much is to be made out, and then the bodies look something like large goblet-cells; but if the tissue be fixed by soaking in Foa's solution immediately on removal from the body, a difference between the normal goblet-cell and these structures is apparent.

> "In the case of trachoma one is struck by the resemblance between these bodies and those oval bodies found in the cavities and epithelial cells in molluscum contagiosum. These latter are by Neisser and Pfeiffer considered to be parasitic protozoa, and the cause of the outgrowths in which they are found."

In 1907 Halberstaedter and v. Prowazek[1] rediscovered Ridley's Trachoma cell-inclusions, and examined them by experiments on the results of which they founded the theory of the "Chlamydozoa," or cloak-covered animalcules—Greek χλαμυς, a cloak. In their opinion the infective matter consists in certain minute red-staining granules in the central parts of the trachoma corpuscle, which is composed of these granules, invested by a covering derived from the nucleolus of the host cell, the nucleolus escaping from the nucleus for this purpose. These authors quote the findings of Négri and Carini to the effect that the Guarnieri bodies can be digested with trypsin or pepsin, and still be infective; this accounting for the filterable quality of the virus. Prowazek's theory has been widely received on account of its originator's high standing among biologists. I have no doubt that if the trachoma bodies are patiently

[1] Ludwig Halberstaedter and S. von Prowazek, "Archiv. a. d. k. Gesundheitsamte," 1907.

studied for vital phenomena on the supposition that they are protozoa, they will give uncontrovertible evidence that they are protozoa, and allow it to be seen that not merely the obscure central part, but the whole corpuscle is a protozoon or a protozoan colony.

The facts recorded in Chapter IV. concerning molluscum contagiosum remove that disease from the nebulous, hypothetical "Chlamydozoa," and I doubt not that the whole theory is unsound. A nucleolus is indistinguishable from a small chromidium; in molluscum the development of the escaped body proves it to be no nucleolus, and the same is true of the Guarnieri bodies, and the protozoa of cancer. Prowazek's escaped nucleolus is as unwarranted an assumption as Virchow's "Vielleicht ein auffallende Kern." It is a narcotising error, which has already diverted some of our ablest bacteriologists to unprofitable speculations on the invisible.

It may be asked whether those to whom we owe the important knowledge we possess of filterable viruses have controlled their results by experiments on the viruses of diseases we know to be caused by parasites which have visible phases. Have, for instance, patient experiments been made with the juice of chancres diluted with blood-serum, suitable

monkeys among other animals being inoculated with the filtrates? If this has been done the record has not come to my notice; and if it has not been done, the omission is a serious one which should be made good. I am strongly inclined to believe that the virus of syphilis will prove to be as filterable as that of molluscum contagiosum. Even the experimental method will lead us into error if we elect to do certain experiments to the exclusion of others.

It is, of course, possible and even probable that there may be protozoan parasites whose largest phase is too small to be visible by our present microscopes, but we should make sure by careful observation and experiment that we do not include objects that are quite obvious with really invisible creatures. The list of so-called "ultramicroscopic protozoa" is already large, as the following quotation from an article by E. A. Cockayne (*Medical Press and Circular*, Feb. 12, 1913) will serve to show:—

"Yellow fever.
Dengue, or break-back fever.
Sandfly fever.
Small-pox (variola).
Cow-pox (vaccinia).
Sheep-pox.
Swine-pox.
Foot and mouth disease.
Cattle plague or rinderpest.
African horse sickness and the nearly allied Catarrhal fever of sheep (both South African).
Pleuro-pneumonia of cattle.
Myxomatosis of rabbits (also affecting dog and man, South America).
Agalasie contagieuse de brebis.

Distemper or 'maladie des jeunes chiens.'

Rabies.

Measles.

Infectious or pernicious anæmia of the horse, in which the blood picture is very like that of pernicious anæmia in man, and suggests that it may also be one of these diseases.

"Trachoma and a non-gonococcal urethritis probably due to the same organism.

"Epithelioma contagiosum of fowls and diphtheria of wood-pigeons, probably forms of the same disease.

"'Farcin cryptococcique.'

"Mumps.

"Anterior polio-myelitis; the polio-myelitis of poultry, of the horse and of the dog are probably allied, but not identical diseases, since the human virus did not affect them, though several species of monkey were susceptible to it.

"A disease (jaunisse) of silkworms.

"Mosaic disease of the tobacco plant.

"Variola of carp.

"Disease of the lips of barbel.

"Cyanolophia gallinarum or chicken typhus and an allied disease affecting three species of thrush and the starling in Italy.

"Passages by means of filtrates have been made through a series of animals in—

Rabies.
Variola.
'Maladie des jeunes chiens.'
Pneumo-enteritis of pigs.
Pernicious anæmia of horses.
Cyanolophia gallinarum.
Thrush epidemic.
African horse sickness.

Foot and mouth disease.
Anterior poliomyelitis.
Epithelioma contagiosum of fowls.
Yellow fever.
Measles."

In chickens spindle-celled sarcoma has been found by Rous to be due to a filterable virus.

CHAPTER XI

THE PROTOZOA OF SYPHILIS AND THEIR RELATION TO THOSE OF CANCER ; AND HOW THE FAILURE OF "IMPERIAL CANCER RESEARCH" TO ESTABLISH THE CAUSE OF CANCER SHOULD BE QUICKLY REDEEMED [1]

THE time has come when a brief, impartial and well-organised inquiry would determine the immediate causation of cancer.

Three years ago some important observations of the intracellular phases of the protozoon of syphilis were published. These observations confirm and extend the demonstration of intra-cellular protozoa in syphilis which I made nearly twenty years ago. The stimulus that led to the new observations was a paper by E. Halford Ross (*Proceedings of the Royal Society*, 1912) on cell-inclusions in the uninucleated leucocytes of the guinea-pig. The best-known form of these cell-inclusions is called Kurloff's body. Ross states that when a guinea-pig's blood is

[1] Reprinted with additions from *The Medical Press and Circular*, March 18, 1914.

examined in the usual way after drying and fixation Kurloff's bodies may easily be passed over as artefacts, but if a drop of fresh blood is placed upon a cover-glass and the latter is inverted upon a slide on which is a layer of specially prepared agar jelly tinted with methylene blue, the bodies take up the stain, and they are then seen to exhibit a series of forms in the last of which a chromatic skein breaks up into spiral segments. Further examination proved that these segments escape as motile spirochæte-like bodies from the parent protozoon. Ross's illustrations show that in several of its phases the Kurloff body is identical in form with the best known of the cell inclusions of both epithelial cancer and sarcoma. Influenced to some extent by the paper just referred to, J. E. R. McDonagh examined syphilitic lesions for intra-cellular protozoa. The best account of McDonagh's observations that I have seen is in a paper in the *Dermatologische Wochenschrift*, April 12, 1913. Some of the forms there depicted as parasites are, I have no doubt whatever, parasitic protozoa, and I can identify some of them with the bodies which I demonstrated to the Pathological Society in October, 1894, in the epidermis at the margin of a secondary syphilitic ulcer (see Fig. 61). The interpretation I put upon these cell inclusions in syphilis was the out-come of close study of protozoa, and it was confirmed

by my finding similar bodies in the epithelial cells of the cornea of a rabbit three days after I had inoculated it with the juice of a chancre (see Fig. 62). This

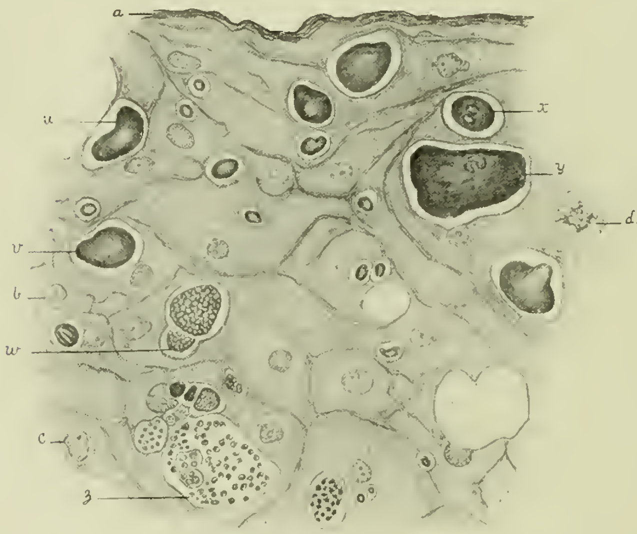


Fig. 61.—Part of the Epidermis near the Margin of a Spreading Secondary Syphilitic Ulcer. (Reduced from a camera drawing made with Leitz's drawing eye-piece and a 1-12-in. oil-immersion lens.) *a*, Horny layer; *b*, normal nucleus of epidermal cell; *c* and *d*, nuclei of epidermal cells that are breaking up; *u*, *v*, *w*, *x*, *y* and *z*, various stages of the bodies described as protozoa by the author in 1894. Two leucocytes are present among the minute bodies at *z*. Compare these protozoa with stages of Didymium, Chap. IV.

observation was confirmed also by E. Pfeiffer (*Centralblatt für Bakteriologie*, 1895). Some of the phases photographed and drawn by McDonagh in

syphilis are identical in form with bodies that I described as protozoa in human cancer and sarcoma and in the infective sarcoma of the dog. E. H. Ross (*British Medical Journal*, December 14, 1912) confirms some of McDonagh's observations in syphilis.

Whenever there is question of protozoa it must be remembered that all the best-known species assume many phases; the life-cycle of one and the

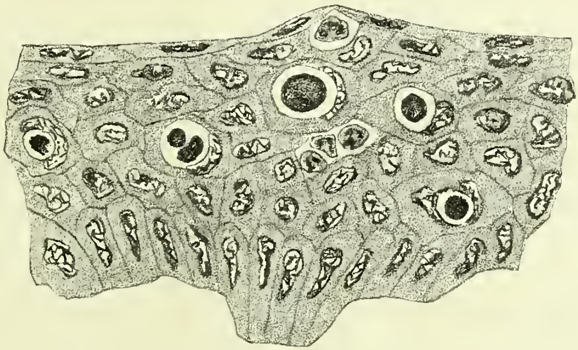


Fig. 62.—Section of a Guinea-Pig's Cornea Three Days after Inoculation with Chancre Juice. × 500 diams. Some of the corneal cells contain inclusions comparable to Guarnieri's corpuscles. From the *Centralblatt für Bakteriologie*, March 15, 1895.

same protozoon may include forms which differ as widely as a sponge, a starfish, and an eel differ one from another in external appearance. The range of forms is further complicated by adaptive and protective changes such as are due to variations of environment, *e.g.*, difference in host-cells, etc. Moreover,

involution forms complicate matters, and, finally, the likeness of some of the host-cells to some of the phases of the parasites may be very close, but the parasites are distinguishable whether by their optical character, their structure and staining reactions, or by their origin, or by their movements.

Different observers may recognise but a part of the life-cycle of the same protozoon present in a disease. It follows that until knowledge is more advanced much caution should be used in naming protozoa and in framing schematic figures representing life-cycles. The latter may even now be helpful if they are understood to be merely provisional. It would be misleading to call the protozoon of syphilis a leucocytozoon, seeing that this disease and also yaws, which is closely akin to it, have many lesions which are mainly epithelial. It is a striking fact that the bodies *u*, *v*, *w*, *x*, *y*, and *z* in Fig. 61 are all homologous with stages of *Didymium difforme* as described above in Chapter IV.

In order to illustrate how the pathology of syphilis may bear on that of cancer, clinical facts and morbid anatomy as well as the histology must be borne in mind. Fig. 63 shows a section of the tongue in which a syphilitic lesion is becoming cancerous. Fig. 64 shows how closely large gummata may resemble malignant growths in a liver. These

familiar objects will serve to give an objective basis to what follows.

We know that typical syphilitic differ from typical cancerous lesions in certain features, but where leucoplakia is becoming changed into cancer these differences are very gradually acquired, and it is not possible either clinically or pathologically to

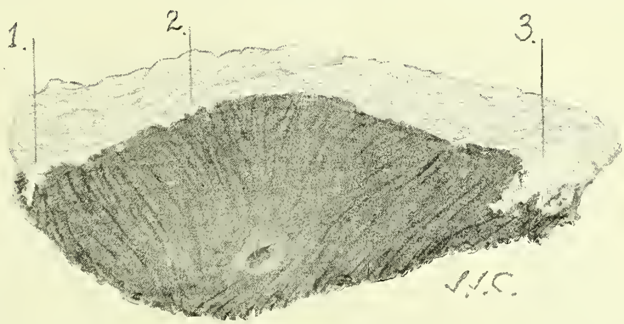


Fig. 63.—A Section through the Middle of a Tongue Removed for Cancer following on Chronic Syphilis. Commencing cancerous infiltration is shown at 1, 2 and 3.

say at what moment one disease becomes converted into the other. Protozoa are equally abundant in both, and they appear to be a series of phases of the same parasite. I do not mean to say that all cancer is a modified form of syphilis, but as far as can be seen it is so in this particular instance. Probably cancer is produced by as many different species of protozoa as there are different bacteria, etc., that cause chronic granulomas. As the protozoon of

small-pox can be so modified that it produces in man only vaccinia and that through endless generations, so the protozoa of syphilis may become so harmonised

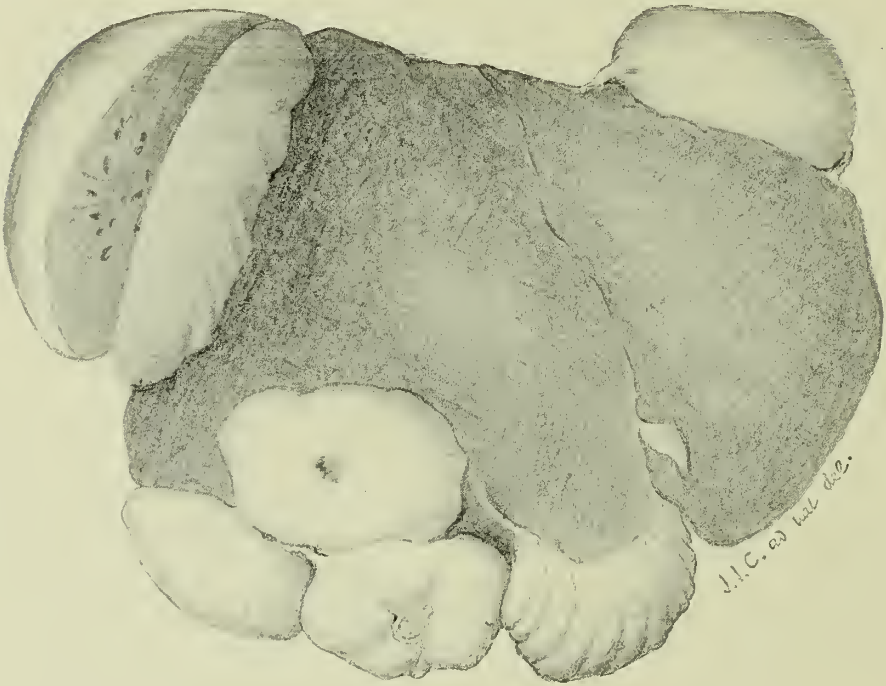


Fig. 64.—The Liver of a Child aged Six Years who died of Hereditary Syphilis. There are five separate gummata and a diffuse infiltration in the neighbourhood of the gall-bladder.

with the cells they infect as to produce only cancer thenceforth. Cancer is a protozoan infection in which a peculiar biological balance is established between the host-cells and the parasites. If the power to

increase in number is about equal among the host-cells and the parasites, the resulting tumour will be such as could be successfully transplanted in suitable soil. The multiplication of tissue-cells is a defensive measure, and may be compared to that defensive autotomy observed in the protozoon *Stylonychia*, which, when seized by an acincta, another protozoon, divided into two, one part swimming away safely, whilst the other part remained as its enemy's prey.

"Cancer Research" has up to the present been completely sterile of results in the direction of finding any cause for cancer. The reason of this is that all the investigations have been done under the assumption that the Virchowian conception of cancer formulated sixty-eight years ago, long before there was any intimate knowledge of parasitic protozoa, is sacrosanct and final.

The difference of tissue type in malignant growths as seen under the lower powers of the microscope and made familiar by Virchow and his successors, and the origin of some cancers in embryonic residues (Cohnheim) are both in harmony with a protozoan causation.

Some of the chief points in Virchow's teaching with respect to cancer are subjoined, and the present position of knowledge with regard to them is appended to each.

1. That the nucleated cell is the biological unit: untrue; protozoa in the chromidial state exist in cancer, and are non-nucleated.

2. That the cells of the tissues surrounding a sarcoma are never converted into cells of the tumour: this is untrue; see Part II. of this work.

3. That cancer and sarcoma are separate diseases: untrue, and has been disproved by epithelial cancer changing into sarcoma after transplantation.

4. That cancer is a specific disease unconnected with any other disease: highly improbable; as stated above, when chronic syphilis of the tongue becomes cancer similar protozoa abound in both the syphilitic lesion and the cancer.

5. That "cells of endogenous origin" belonging to the victim occur in cancer: untrue, and disproved by an observation which I made in 1901 and recorded in 1907; see Fig. 50, p. 132. Such cells are daughter parasites which arise by free cell-formation within protozoa in the chromidial condition.

Those who still adhere to Virchow's teaching are constantly forced to explain away features they do not understand in cancer by assuming without critical investigation that they are due to degeneration or phagocytosis.

E. F. Bashford (*Reports of the Cancer*

Research Fund, 1905) has made a firm stand by the second of the points mentioned above, and it is of vital importance. I have disproved it by minute examination of typical sarcomas, *e.g.*, of the breast, and my view has recently been independently confirmed by McDonagh in a paper entitled "Some Transformation-forms of Plasma-Cells," published in the *Archives für Dermatologie und Syphiligraphie*, vol. cix., Heft 3, S. 441. McDonagh found close to the blood-vessels of a round-celled sarcoma that the lymphocytes were converted into plasma-cells. Farther from the blood-vessels the plasma-cells were found to merge into those of the sarcoma, and to be loosely arranged with indefinite cell-membranes; and their nuclei possessed but little chromatin, but contained bright transparent "nucleoli." The gradual change of the plasma-cells into sarcoma cells was clearly traced. From the illustrations which accompany this paper, I have no doubt that if the sections are examined again and the parts of the tumour still farther from the blood-vessels are very slowly and carefully studied under an oil-immersion lens, some of the large "nucleoli" will be found to have been in the act of escaping from the remains of the nucleus, others, grown larger, to be lying in the cytoplasms, others again to be free and to be subdividing into young broods with or without the

appearance of points of chromatin or definite nuclei. They are protozoa and the cause of the tumour. Their earliest intranuclear stage is probably invisible as is Plasmodiophora, the protozoon that causes club-root in the cabbage, when it first enters the cell cytoplasm. When from their appearance they simulate nucleoli these protozoa of sarcoma are in the chromidial condition. The chief features of the chromidial phase of protozoa are sketched above in Chapter VI. of this book; here I may repeat that in this state the animalcule either contains no separate nucleus or but part of that which it may have had previously, chromatin having wandered from the nucleus and as "chromidial dust" mixed intimately with the cytoplasm. Every particle of such a protozoon contains both a nuclear and a cytoplasmic element, and has all the potential of a cell. The filter-passing property of the viruses of certain diseases, such as small-pox and rabies, is thus simply explained, and the strained hypothesis that underlies the name *Chlamydozoa* is made unnecessary, and has proved to be untrue in the case of molluscum contagiosum. Chromidial protozoa may form internal or external buds or gemmules, themselves in the chromidial state; or, in favourable conditions, a chromidial protozoon can produce new nuclei by free-nucleus formation. Such new nuclei

may be either quite rudimentary or very highly organised, and may be produced either at the surface or in the interior of the parent cell, and they constitute independent new cells by separating themselves from the parent cell together with a portion of cytoplasm. When such a new protozoon is formed inside the parent we have what Virchow termed a "cell of endogenous origin." The peculiar protozoa of cystic ureteritis, by close study of which, in 1891, I was first enabled to recognise protozoa in cancer, are in this chromidial condition, as are also the protozoa of molluscum contagiosum. Such protozoa closely resemble colloid, hyaline, and other degeneration-products for which they are mistaken. Once the true nature of these chromidial parasites is recognised, the patho-biology of some cancers and sarcomas can easily be studied in a single small section under a 1-12th-inch oil immersion lens.

The death-rate due to cancer is said to be increasing. According to R. Hingston Fox (*Lancet*, January 17, 1914), it now accounts for one in every nine deaths among all adults of twenty-five and upwards, and for one out of every five deaths in women between the ages of forty and sixty. These terrible figures probably do not include the blood-sarcomas, leucocythæmia, and the like; and the deaths from sarcoma in children and young people are

not few. Is it, then, not time for us to reconsider our responsibility as a profession ?

I am convinced that a conference organised on broad principles, such as I suggest below, would enable the enigma to be solved. In preparation for such a conference all pathologists who have not already done so should familiarise themselves with mycetozoa and common parasitic protozoa, such as abound in every earthworm and every cockroach. Pathogenic protozoa are so widely distributed in Nature that, given the protozoan origin of human cancer, it would be surprising if kindred parasites did not cause a similar disease in, say, the mouse, the trout, and the cod.

The cell-inclusions of the vaccinated cornea in rodents should be very carefully studied, especially from the third to the sixth day, and in relation with them the chromidial state, which has hitherto been seen and studied chiefly in certain rhizopods. The conference should be initiated by inviting all who have specimens, microscopic or macroscopic, that may have a bearing on the question of protozoa in cancer or other diseases to send a descriptive list of a stipulated number of such for exhibition in some central place in London, where they would be received by the agent of a committee who would be responsible for them, and who would arrange for their proper

exhibition. A fully illustrated catalogue of all the specimens should be prepared, published and sold at not more than cost price a month at least before the opening of the exhibition. The latter should be made easy of access, and should be open in the evenings as well as by day, and continue open for at least a month. At the end of that time a discussion should take place in a room where the specimens are on view; the rules for this discussion should be published with the catalogue. Those desiring to speak should be required to send in their names by a certain day, and the order in which they are called on should be decided by drawing lots. The chairman at the debate ought not to be committed to any personal views on the subjects under discussion, and he ought to limit his duty to seeing that the speakers adhere to the rules. One of the rules should be that speakers must confine their remarks to direct criticism of the objects exhibited. If any objective feature thus referred to had not been illustrated in the catalogue, it should be drawn or photographed and embodied with a verbatim report of the whole debate to be published and sold in the same way as the catalogue. Only in such a way as I here roughly suggest can a sound and impartial judgment be formed on visible objects so widely spread and numerous as those in question.

If such a scheme were adopted, I am confident that in a relatively short space of time the conviction that cancer is caused by protozoa would prevail, and thus the failure of Imperial "Cancer Research" could be quickly redeemed, and a new and fruitful era in Pathology and Medicine would be inaugurated.

CHAPTER XII

SOME RHIZOPODS AND A CHYTRIDIAN OCCURRING WITH "DIDYMIUM DIFFORME"

In course of watching cultures of Didymium I have observed many other protozoa and some organisms which I take to be Chytridians. The rhizopods are most abundant during the first few days after the material is brought from the garden and put in a moist chamber or in an open culture dish such as that shown in Fig. 12; the Chytridian I have noticed only in cultures some weeks old, in which bacterial growth is abundant.

Some of these Rhizopods may be mentioned here to indicate in a measure the associates of the Mycetozoon.

The amœba shown in Fig. 65, *a*, was found on several occasions in the same material that contained large encapsuled forms (Fig. 18). The character of their nucleus does not exclude the possibility of their belonging to the same cycle of forms, but since I have not actually seen them become encapsuled,

I have not described them as belonging to the Mycetozoon.

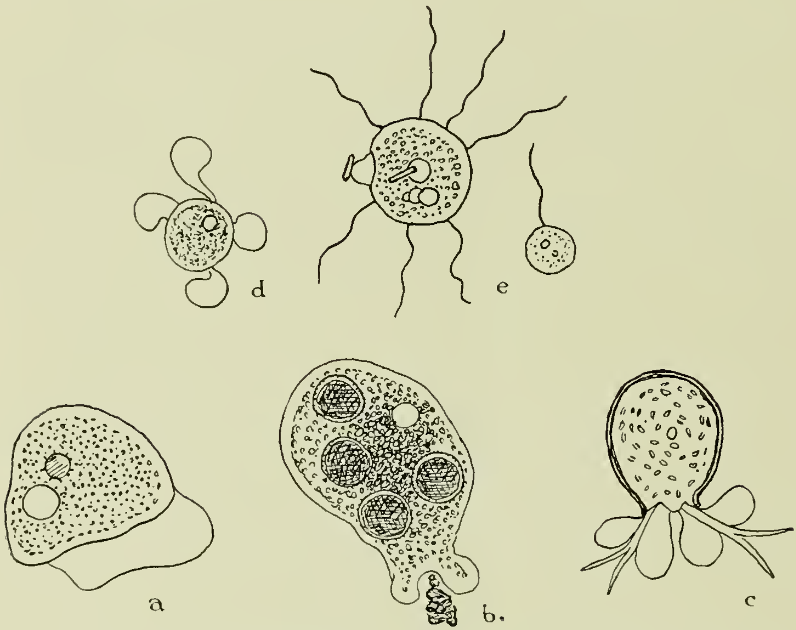


Fig. 65.—*a*, Large amœba with a nucleus like that seen in some phases of Didymium; *b*, an amœba of the proteus type; it has ingested four Didymian spores, and is in process of ingesting a clump of chlorophyll granules; *c*, a shelled amœba with both stiff and lobose pseudopodia; *d*, a small amœba; its pseudopodia were protruded explosively at intervals of about one second; *e*, a round protozoon with a single flagellum and a multiciliate amœba in the act of capturing a bacillus: the multiciliate form may arise by a fusion of organisms having the single flagellum.

Several other rhizopod forms besides those shown in Fig. 65 were observed.

Chytridiæ.—A. Lister mentions the suggestion that the mycetozoa may be related to the Chytridiæ, which do not always form a mycelium, and in which the entire vegetative body is finally transformed into a many-spored sporangium, the vegetative body and spores having the power of

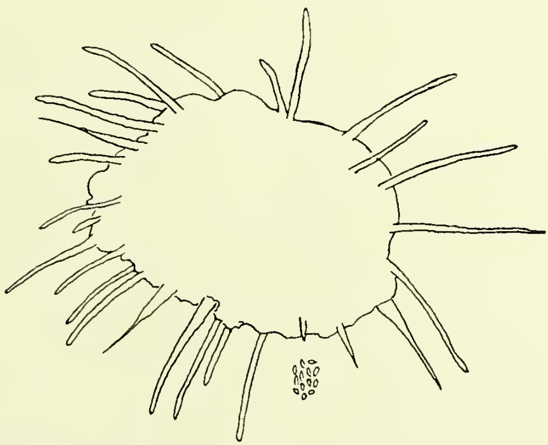


Fig. 66.—A Chytridian Growth. A surface view. × 100 diams.

amœboid movement for a longer or shorter time. De Bary mentioned, among other points of difference, that the Chytridiæ do not form a plasmodium by coalescence of swarm-cells, "and there is therefore no ground to assume their direct relationship with the Mycetozoa." The occurrence of Chytridian elements in cultures of Didymium has been

mentioned in Chapter I. It appears to affect old cultures. In that shown in Fig. 12, after four weeks very minute white points appeared on one of the tea-leaves. From the central roundish point fine white

FIG. 67.—PHASES OF CHYTRIDIANS. *a*, Mycelium with terminal spores and part of a large segmented rod; *b*, *c*, two Chytridian tubes, one segmented and containing spores; *d*, spore-cyst; *e*, (?) spore of Didymium; *f*, *g*, Chytridians developing in Euglena, the nucleus of which is centrally placed; *g*, Chytridian tubes growing out from the host-cell.

rods radiated, as shown in Fig. 66. On teasing out one of these white bodies and examining the preparation, the structure shown in Fig. 67, a, was obtained; this I took to be the mycelium of a Chytridian. Some of the long segmented rods contained spores;

other filaments showed vital activity in the presence of motile globules in their protoplasm. Having no botanical work which deals with Chytridians at hand, I am uncertain whether they invariably begin their career as cell-parasites. The diagrams (Fig. 67, f and g), which I made on reading an article[1] some years ago, show the intracellular stages of a Chytridian, the host being the large flagellate Euglena, common in ponds.

In teasings of these Chytridian foci structures the size of Didymium spores occur with nuclei like that of the mycetozoon spore, save that it has near it a granular structure, and the protoplasm is uniform instead of granular; whether these are spores infected by the Chytridian I cannot say with the limited experience that I have of them at present.

[1] Kurt Nägler, "Chytridiaceæ in Euglena," *Archiv. für Protisten Kunde*, October 12, 1911.

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THE END

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